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Estimation of total serum fucose in patients with end-stage renal disease

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Abstract:

The objective of the study: is to compare between concentration of total serum fucose in normal healthy controls and patient with end-stage renal disease**.**

Design: cross-sectional study of patients with end-stage renal disease **Setting:** Al- Kut hospital Department of renal dialysis in Al- Kut city. **Patients and Method**:-This cross-sectional study incorporated patients with end-stage renal disease (October 2011 to September 2012) in Al- Kut hospital Department of renal dialysis.

Results: The patients were arranged into two groups, group 1 (normal healthy controls) and group 2(patients with end-stage renal disease). Regarding group 1 (which were 29 individual). Regarding group 2 which represents the patients with end-stage renal disease (there were 25patients).the concentration of total serum fucose was increased in patients with end-stage renal disease and also we found that there is positive relationship between the level of the total serum fucose and the serum creatinine and blood urea .

Conclusions: the level of total serum fucose increase in patients with end-stage renal disease and this level have positive relationship with level of serum creatinine and blood urea.

Introduction

End-stage renal disease represents a stage of chronic kidney disease where the accumulation of toxins, fluid, and electrolytes normally excreted by the kidneys results in the uremic syndrome, This syndrome leads to death unless the toxins are removed by renal replacement therapy, using dialysis or kidney transplantation. [1,2]

The pathophysiology involves two broad sets of mechanisms of damage: (1) initiating mechanisms specific to the underlying etiology (e.g., immune complexes and mediators of inflammation in certain types of glomerulonephritis, or toxin exposure in certain diseases of the renal tubules and interstitium); and (2) a set of progressive mechanisms, involving hyperfiltration and hypertrophy of the remaining viable nephrons, that are a common consequence following long-term reduction of renal mass, irrespective of underlying etiology.[2,3] The responses to reduction in nephron number are mediated by vasoactive hormones, cytokines, and growth factors. Eventually, these short-term adaptations of hypertrophy and hyperfiltration become maladaptive as the increased pressure and flow predisposes to sclerosis and dropout of the remaining nephrons, Increased intrarenal activity of the renin-angiotensin axis appears to contribute both to the initial adaptive hyperfiltration and to the subsequent maladaptive

hypertrophy and sclerosis, the latter, in part, owing to the stimulation of transforming growth factor (TGF-), This process explains why a reduction in renal mass from an isolated insult may lead to end-stage renal disease over many years.[2, 3.] The symptoms of chronic kidney disease often develop slowly and are nonspecific; Individuals can remain asymptomatic until renal failure is far advanced (GFR < 10–15 mL/min). Manifestations include fatigue, weakness, and malaise, Gastrointestinal complaints, such as anorexia, nausea, vomiting, a metallic taste in the mouth, and hiccups are common, Neurologic problems include irritability, difficulty in concentrating, insomnia, subtle memory defects, restless legs, and twitching, Pruritus is common and difficult to treat.[2,3,4] As uremia progresses, decreased libido, menstrual irregularities, chest pain from pericarditis and paresthesias can develop. Symptoms of drug toxicity especially for drugs eliminated by the kidney increase as renal clearance worsens.[5,6, 7, 8]

L-Fucose is a methyl pentose sugar similar to galactose except for the loss of the alcohol group on carbon number 6, it is found in human blood serum bound to protein by a covalent bonding and it is part of a large group of compounds known as glycoproteins.[21, 22]
L-Fucose (6-deoxy-L-galactose) is a monosaccharide that is a common component of many N- and O-linked glycan and glycolipids produced by mammalian cells, Two structural features distinguish fucose from other six-carbon sugars present in mammals, These include the lack of a hydroxyl group on the carbon at the 6-position (C-6) and the L-configuration- Figure (1).[23]

It is also found at the terminal or pre-terminal position of many cell surface oligosaccharides ligands that mediate cell reorganization and adhesion signaling pathways.[24]In mammals, fucose-containing glycans have important roles in blood transfusion reactions, selecting-mediated leukocyte-endothelial adhesion, host-microbe interactions, and numerous oncogenic events, including signaling events by the Notch receptor family, Fucose metabolism appears to be altered in various diseases, Several studies have concluded that Fucose metabolism is abnormal in several pathological processes like cystic fibrosis, diabetes, cancer and atherosclerosis. [23,25]

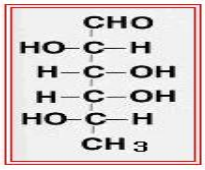

Figure (1): L-Fucose structure

Elevated levels of serum protein-bound Fucose have been reported in patients with malignant disease and in certain benign disorders.[14,15]
Markedly, elevated Fucose levels have been assessed in cancer of the breast,[16,17,18,19] gynecological cancers, leukemia,[20] small cell lung cancer, and ovarian cancer.[14,21,22,23] Abnormal metabolism of L-Fucose in Hepatocellular carcinoma was observed. [24] An increase in Fucose levels was also observed in cardiovascular disorders[25] and in patients with depression.[26]

Aims of the study
1. To evaluate and compare serum Fucose levels in healthy controls and patients with end stage renal failure.

2. To ascertain the role of serum Fucose as an effective parameter for follow up patients with end stage renal failure

Materials and Methods

Fifty four human formed the case material for this study arranged into two groups, group 1 (normal healthy controls) and group 2(patients with end-stage renal disease). regarding group 1 (which were 29 normal individual). Regarding group 2 which represents the patients end-stage renal disease (there were 25patients).

Exclusion Criteria: Those with following condition excluded from this study: malignant disease, leukemia, depression, rheumatoid arthritis, coronary artery diseases, & immunological diseases.

Sample collection

Blood was collected by venipuncture from the patients .The samples were collected on different days because of patients visiting hospital on different days. The serum was separated, centrifuged and stored at -20°C.

CHEMICALS:

All common laboratory chemicals and reagents used in this study were of Analar grade unless otherwise specified and were obtained from the following companies: BDH company ;(UK). H_2SO_4 , L-cysteine ., Biomaghreb company ; total protein kit, & Sigma company ;(USA). L-Fucose.

STATISTICAL ANALYSIS:

Students t-test was used to determine if the mean value for biochemical tests were significantly different in the normal healthy control and head and neck tumor, $p< 0.05$ were considered significant.

SERUM TOTAL FUCOSE DETERMINATION :

The principle of this method depends on the formation of chromogen on addition L-cysteine to test tube contains the sample and sulphoric acid · The color product formed by Fucose has maximum absorption at 396 nm, and almost no absorption at 430 nm. Reagent (1) : Sulphoric acid solution ,prepared by addition one part of dis. water to six part of concentred sulphoric acid (V/V). This reagent is stable for many years in room temperature. Reagent (2): L-cysteine hydrochloride solution: made by dissolving 3grams of cysteine in 100ml of dis. water. This reagent is stable for one week in 2-8 C^o.

C. PROCEDURE:

The reaction was performed in 18×150 mm Pyrex test tube labeled as test , standard, test blank , and standard blank, into which the following were pipette as the following:

Reagent	Test	Test blank	Standard	Standard blank
Sample	20µl	20µl	---	---
Standard	---	----	20 µl	20µl
Reagent (1)	4.5ml	4.5ml	4.5ml	4.5ml

Heating for 10 min. in boiling water bath, after that finally cooled in tap water.

Reagent(2)	100 µl		100 µl	
D.W		100 µl		100 µl

Let Stand for 2hr. and after that reading into wave length 396nm—430nm

D. CALCULATION :

Total serum Fucose (mg/dl)[2].

Expressed as following =

$$\frac{(At(396)-At(430))-(Ab(396)-Ab(430))}{(As(396)-A\square(430))-(Ab(396)-Ab(430))} \times \text{concentration of standard}$$

=mg/dl

Where :

At= Absorbance of the test solution at(396,430nm)

As=Absorbance of the standard solution at(396,430nm)

Ab= Absorbance of the blank solution without cysteine at(396,430nm)

MEASURMENT OF SERUM TOTAL PROTEIN:

Cupric ion in an alkaline medium interaction with protein peptide bonds resulting in the formation of a coloured product whose absorbance is measured at 546nm[3] . Reagent (1): Alkaline reagent composed of 12 mmol/l Sodium- Potassium tartrate , 0.6mmol/l NaOH, and 30mmol/l Potassium iodide .Reagent(2) : Biuret reagent ,0.6 mol/l hydrate copper sulphate ($CuSO_4$) Colouring reagent. Reagent (3): standard of total protein 5g/dl. Working reagent : 3ml R(2) add to 147 ml R(2) , stable for 6 months at+2 -8 ^{0}C .

C. PROCEDURE: The reaction was performed in 18×150 mm test tube labeled as test , standard, and blank, into which the following were pipette as the following:

Reagent	Test	Standard	Blank
Sample	20µl	----------	----------
Standard	----------	20µl	----------
Working reagent	1ml	1ml	1ml

Mixed well after each addition and let stand in room temperature for 5 min(+20,+25). The absorbance read at 546 nm.

D.CALCULATION:

$$\text{Total protein (g/dl)} = \frac{At - Ab}{As - Ab} \times \text{concentration of standard}$$

Where:

A_t =the absorbance of the test solution at 546 nm

A_s=the absorbance of the standard solution at 546 nm

A_b= the absorbance of the blank solution at 546 nm

Results :-

Total serum Fucose level was measured in patients with renal disease and the result revealed that the TSF increased in patient with renal disease in compared to normal healthy controls P<0.0005. As showing in table (1) and figure (2)

Table (1): Biostatistical calculations and student and t-test for total serum Fucose in sera of normal healthy controls and patient with renal disease.

TSF (mg/dl)	Normal healthy controls	Renal disease
Sample size	N1=35	N2=25
Means	X1=12.46	X2=16.1
Standard deviation	SD1=3.64	SD2=5.67
Standard error of the mean	Sx1=0.67	Sx2=1.134
Confidence interval of the mean	11.1-13.81	14-16.3
t-test		2.06
Probability		P<0.0005

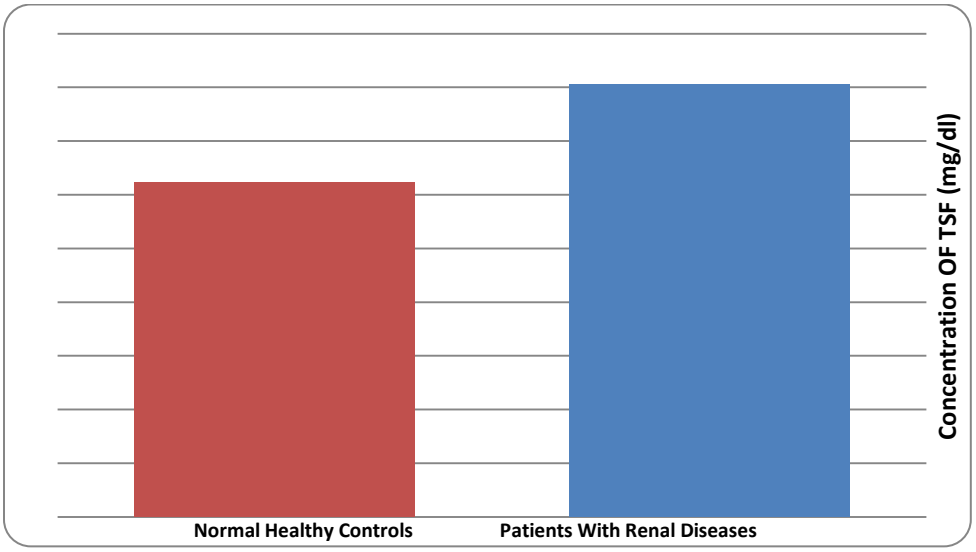

Figure (2)show concentration of TSF in patients with renal dialysis and normal healthy control .

\When we studied the level of total serum protein in sera of normal healthy controls and patient with renal disease, there was decrease the level of total serum protein in patients with renal disease P<0.0005 as showing in Table (2) and Figure (3)

Table (2): Bio statistical calculations and student and t-test for total serum protein in sera of normal healthy controls and patient with renal disease.

TSP (g/dl)	Normal healthy controls	Renal disease
Sample size	N1=29	N2=25
Means	X1=7.9	X2=4.89
Standard deviation	SD1=0.69	SD2=0.88
Standard error of the mean	Sx1=0.12	Sx2=0.177
Confidence interval of the mean	7.64-8.15	4.5-5.24
t-test		14
Probability		P<0.0005

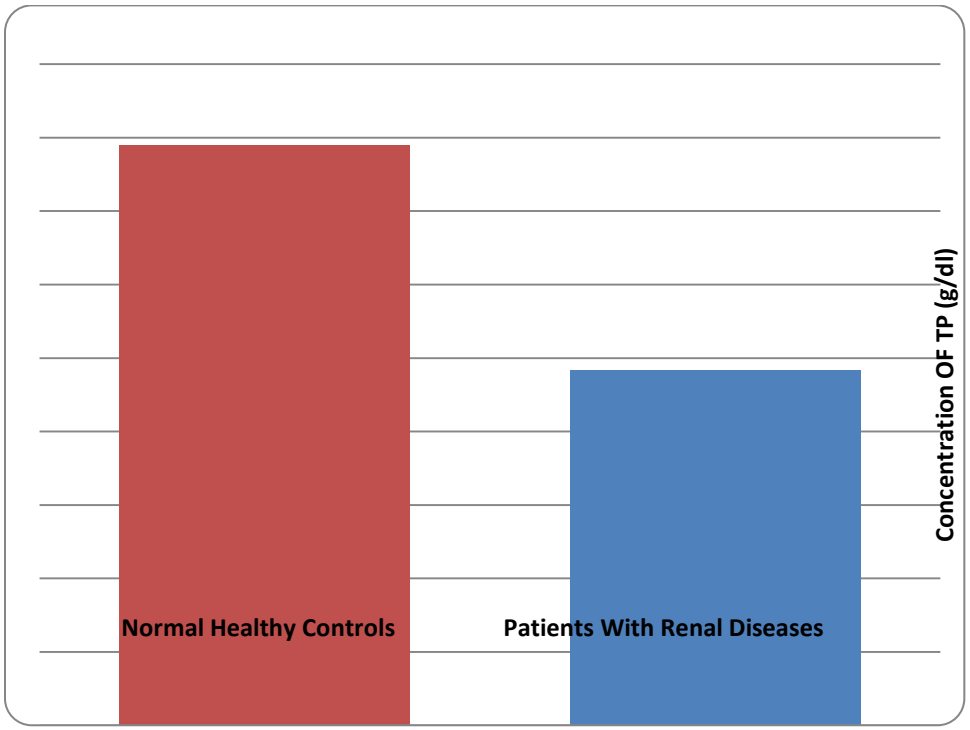

Figure (3) show concentration of TP in patients with renal dialysis and normal healthy control

When we studied the ratio of TSF/TP for normal healthy controls and patient with renal disease we found that this ratio is increased to about the twice times that for normal healthy controls as showing in Table (3) and figure (4)

Table (3): Bio statistical calculations and student and t-test for TSF/TP ratio in sera of normal healthy controls and patient with renal disease .

TSF /TP ratio	Normal healthy controls	Renal disease
Sample size	N1=29	N2=25
Means	X1=1.58	X2=3.1
Standard deviation	SD1=0.455	SD2=1.58
Standard error of the mean	Sx1=0.085	Sx2=0.277
Confidence interval of the mean	1.41-1.75	2.5-3.65
t-test		4.9
Probability		P<0.0005

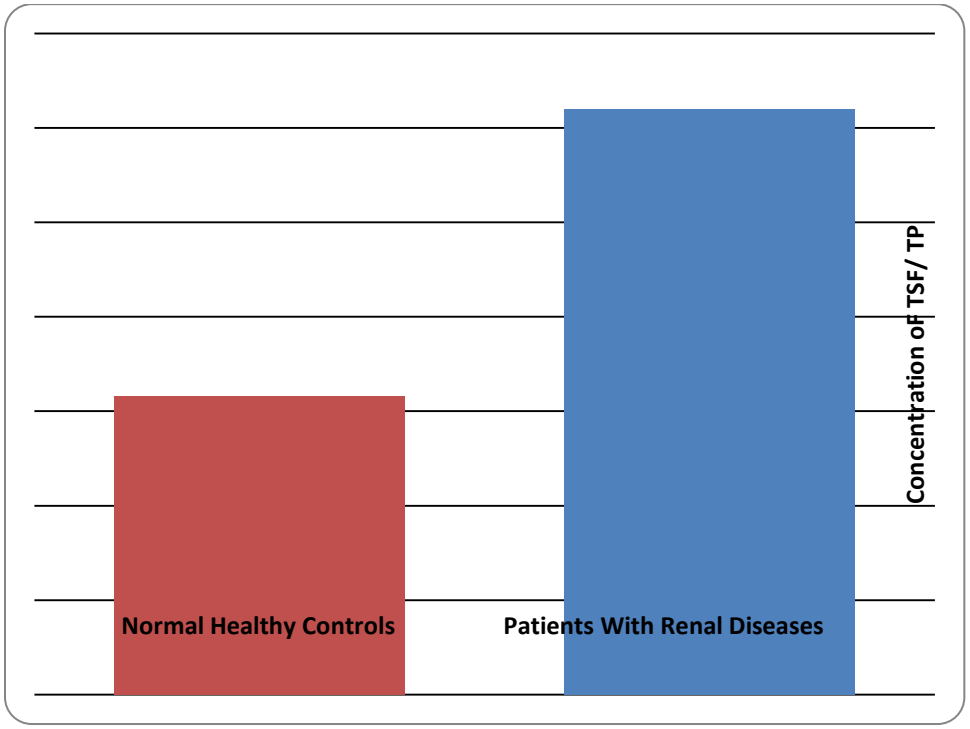

Figure (4) ratio of TSF/TP in patients with renal dialysis and normal healthy control

In our study also we found that there is positive relationship between the level of the total serum fucose and the serum creatinine and blood urea as showing in figure (5) and figure (6)

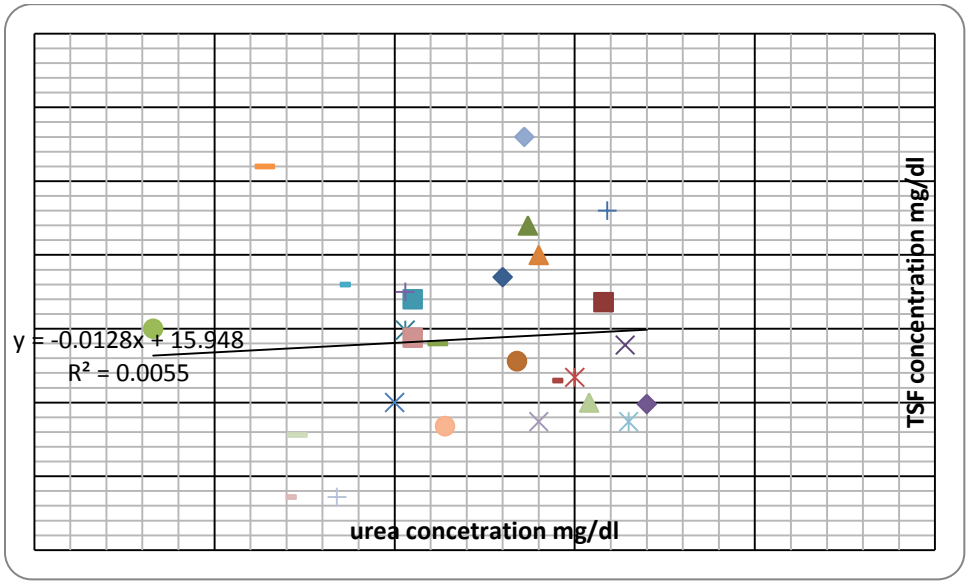

Figure (5) relationship between the level of the total serum fucose and blood urea

positive

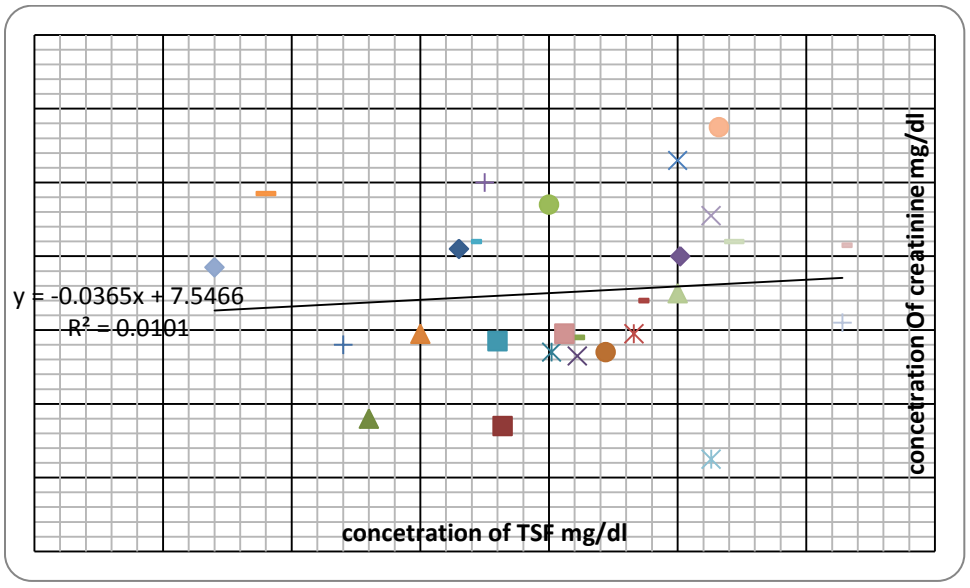

Figure (6) relationship between the level of the total serum fucose and the serum creatinine

DISCUSSION

In our present study, total serum Fucose level was measured to evaluated its usefulness in diagnosis and follow up of patient with renal disease and the result revealed that the TSF increased in patient with renal disease in compared to normal healthy controls P<0.0005.

This is similar to another study report that total serum Fucose level increased in chronic diseases which cause reperfusion injury following ischemia, asthma, and inflammatory skin diseases, are conditions that result from the excessive and uncontrolled recruitment of leukocytes[27]. Also have been detected in rheumatoid arthritis patients.[28]
In our study we found that the ratio of TSF/TP for patients with renal disease we found that this ratio is increased to about the twice times that for normal healthy controls (P<0.0005), and this not discussed before in previous studies

Also we found that there is positive relationship between the level of the total serum fucose and the serum creatinine and blood urea, and this is of medical significance where this can be used to follow patients with renal diseases and can be regarded as another parameter for follow up patients with renal diseases and this also not discussed before in previous studies.

Conclusion:

The rise in total serum Fucose level in patients with renal diseases suggests that it may be used as a general parameter to evaluate renal function in addition to other parameter for those patients with renal diseases.

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