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Transforming growth factor-beta in nephrotic syndrome and its Correlation with Albuminemia and Hyperlipidemia.

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Abbreviations
***TGF-β**, Transforming growth factor-beta*
***NS**, Nephrotic syndrome*

ABSTRACT

Background: Nephrotic syndrome (NS) is one of the most commonly encountered glomerular diseases in children and a major contributor to the workload of pediatric nephrologists. Nephrotic syndrome is a chronic disease that is well known for its long term serious complications. among these complications albuminemia and dyslipidemia. Many studies has related albuminemia and dyslipidemia to other complications of Nephrotic syndrome as retinopathy and cardiovascular disease of nephrotic syndrome. **Objectives**: The main objective of this study was planned to elucidate the potential diagnostic role of transforming growth factor-beta (TGF-ß) as a novel biomarkers and its correlation with albuminemia and dyslipidemia in nephrotic syndrome among Egyptian children. **Methods:** Serum level of TGF-ß was measured using ELISA, serum level of lipid profile and urinary proteins were estimated. **Results:** Our study revealed that serum level of TGF-ß was higher in nephrotic cases than controls and its serum level was significantly negative correlated with serum albumin while it showed a significant positive correlation with urinary protein and lipid indices levels in children with nephrotic syndrome ($r = -0.88$, $P < 0.001$) , ($r = 0.85$, $P < 0.001$) and ($r =$, $P < 0.001$) respectively. **Conclusions:** our data confirmed that TGF-ß can serve as a potential diagnostic biomarker in nephrotic syndrome. Furthermore, it can assist in management of nephrotic syndrome pathogenesis due to its correlation with dyslipidemia and albuminemia which are the main clinical features of nephrotic syndrome.

INTRODUCTION:

Nephrotic syndrome (NS) is a cluster of symptoms including proteinuria, hypoalbuminaemia, and edema. [1] It is the most common chronic kidney disease in children with an estimated annual incidence of 2–7 per 100,000 children and a cumulative prevalence of 16 per 100,000 children.[2] Disturbances of lipid metabolism found in NS have been reported to persist even during remission periods and may predispose to endothelial

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dysfunction or atherosclerosis.[3] Nephrotic patients are prone to develop complications such as hypovolemia, peritonitis, thromboembolic complications secondary to hypercoagulability, and renal impairment.[4] Proteinuria causes increased tubular synthesis of macrophagic and other chemokines, with increased tubular cellular proliferation and apoptosis, leading to interstitial inflammation and fibrosis. [5] TGF-β has been known as a key mediator in extracellular matrix formation. [6] In fibrosis and in tissue remodeling during disease progression in different organs, Up-regulation of TGF-β is informed to be necessary, [7] in which glomerular fibrosis in the kidney is included. [8] Transforming growth factor-β (TGF-β) levels and signaling are enhanced in renal cells during the progression of diabetic nephropathy. TGF-β plays a key role in mesangial cell fibrosis under diabetic conditions by inducing the expression of extracellular matrix proteins such as collagen. [9]

The aim of the present study was to investigate the potential diagnostic role of TGF-ß as a novel biomarkers and its correlation with albuminemia and dyslipidemia in nephrotic syndrome among Egyptian children.

II. Subject and Methods

This study was approved by the ethics committee of Zagazig University (no.3337). The Study included 50 patients with idiopathic NS with an age range between 5 and 9 years (28 males and 22 females) who were diagnosed clinically and proven by laboratory results. These patients had been followed up at the nephrology unit of the Department of Pediatrics, Zagazig University Hospital. The control group consisted of 50 healthy children (28 males and 22 females) selected from the general pediatric outpatient department. Demo-graphic data of the patients including serum albumin and serum urea and serum creatinine were recorded from the medical history and electronic files.

After twelve hours fasting, 3 ml blood samples were withdrawn by vein puncture from the anti-cupital vein and then the blood samples were aliquoted in anticoagulant free tubes, kept at room temperature for 30 minutes until clot formation, then sera were separated by centrifugation at 3000 rpm aliquoted and kept freeze at -20°C until use for determination of TGF-β and lipid profile.

A 24 h urine was collected in plastic tubes from all participating individuals for detection of Total protein and centrifuged (2500g for 10 min), then stored at -70 °C. Immediately before protein assay, the samples were thawed at room temperature.

II.1. lipid profile detection methods: Triglyceride (TG) was measured by lipoprotein lipase peroxidase according to Fossati method[10], Total cholesterol (TC) by cholesterol oxidase methodology according to Allain method[11], high density lipoprotein-cholesterol (HDL-C) by phosphotungstate magnesium chloride methodology according to Lopes-Virella method [12], very low density lipoprotein-cholesterol (VLDL) and low density lipoprotein (LDL-C) levels were calculated indirectly by means of the Friedwald formula[13] as (VLDL-C = TG/5 provided TG = 400 mg/dL) and LDL-C = TC – (HDL-C + VLDL-C).

II.2. TGF-β detection method: Serum levels of TGF-ß were measured by an enzyme-linked immunosorbent assay (ELISA) kit provided from MyBioSource. Inc., California, San Diego, USA, **(Cat No. MBS266143)** and was applied according to instructions given by the manufacturer as the following [14]**:** Double Antibody Sandwich ELISA Technique was applied. The TGF-β antibodies and solid phase carriers were connected to form immobilized antibodies. The uncombined antibodies and impurities were washed out. Combination occurs between antigens and antibodies on carriers into the antigens complex. Combination between TGF-β antibodies with the antigens on immune complexes

occurs by addition of biotin labeling antibodies. The enzyme amount on the carrier is now positively related to the amount of the TGF-β in specimens

II.3. Urinary total protein detection method: The samples were processed using Urinary protein assay kit provided from Creative Bio Mart. Inc. Shirley, USA. **(Cat. No. 0731).** the linear detection range of 5 – 200 mg/dl [15]

III. Statistical Analysis:

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 20. Data are presented as the mean± standard deviation (±SD), Comparison of continuous data was performed using student t-test. The correlation coefficients were calculated using Spearman correlation (because it is a correlation between one parameter and one another parameter i.e. TGF&LDL but multiple regression analysis use correlation between one parameter with many parameters i.e. TGF& dyslipidemia) . P value of < 0.05 was considered statistically significant. [16]

IV. Results

IV.1.1. Lipid profile indices (Dyslipidemia): Our study showed that the mean values of TC (404.3±55) mg/dl, LDL-C(379.4±56) mg/dl and TG (463±93) mg/dl show significant increase in patients with NS compared to control group (126.1±2.3) mg/dl, (63.5±6.7) mg/dl and (120±17.8) mg/dl. As shown in **Table (1)**

IV.1.2. Serum TGF-β level: the mean values of TGF-β declared significant increase (160.5 ± 28.8) pg/ml in patients compared to controls (56.3 ± 25.9) pg/ml. As shown in **Table (1)**

IV.1.3. Urinary protein level (Proteinuria): the mean values of urinary protein declared significant increase (1.45 ± 0.2) mg/dl in patients compared to controls (0.17 ± 25.9) mg/dl. As shown in **Table (1)**

IV.1.4. Correlation between Serum TGF-β and Albuminemia & Proteinuria: We further clarified the relations between the serum levels of TGF-β with Albuminemia and Proteinuria among the nephrotic cases using Spearman correlation analysis. As shown in **Figures (1&2)**, The TGF- β serum levels showed a significant negative correlations with serum albumin (albuminemia) (r= -0.88, P< 0.001*) while TGF-β showed significant positive correlation with urinary protein (proteinuria) (r = 0.86, P< 0.001*).

IV.1.4. Correlation between Serum TGF-β and Dyslipidemia: Serum levels of TGF-β showed significant positive correlation with serum LDL-c, TGs and TC level among the nephrotic patients. As shown, **Figures (5, 6and 7)** (r = 0.84, 0.82, 0.83) respectively, and all with P-value <0.001.

V. Discussion

Despite several advances in NS pathophysiology**,** Clinical evolution is still unpredictable. No cohort study has succeeded in bringing out prognostic factor. Many studies support that TGF-β is a major contributor to glomerular sclerosis and interstitial fibrosis, which are common features of progressive injury in glomerular diseases [17, 18]**.** Our results proved that there was a significant increase in TGF-β level in patients with NS than those in controls, and also a significant negative correlation with albuminemia while our results revealed a significant positive correlation between serum TGF-β and proteinuria and Dyslipidemia.

In accordance with our data, ***Xiaoyi, et al.*** reported that TGF- β level was significantly increased Diabetic Nephropathy Patients [19].This result was expected by another study which identified TGF- β as being up regulated during the course of progressive renal injury [20].

Our results also are in line with another study of ***Katsumi, et al.*** [21] in IgA nephritis and focal glomerulosclerosis which showed a significant increase in urinary TGF-β in patients and paralleled the grade of interstitial fibrosis. Other recent studies declared that Transforming growth factor-β (TGF-β) levels and

signaling are enhanced in renal cells during the progression of diabetic nephropathy (DN). TGF-β plays a key role in mesangial cell fibrosis under diabetic conditions by inducing the expression of extracellular matrix proteins such as collagen [22, 23]. Albuminuria had been investigated as a marker of kidney status in many forms of kidney disease (i.e diabetic nephropathy, chronic renal disease, nephrotic syndrome, etc.) [24]. Also, Another study revealed that micro albuminuria is often present at diagnosis in adults with type 2 diabetes mellitus, reflecting the association between insulin resistance and albuminuria [25] . Our study illustrated a significant negative correlation between albuminemia and TGF-β and the incidence of NS. In Accordance with our results, ***Kinugasa and his colleagues*** found that podocytes contain increased amounts of albumin in kidney biopsies obtained from patients with nephrotic syndrome as well as in animals with heavy proteinuria [26]and also ***Kayo ,et al*** contribute the increase level of albumin in urine of patients with nephrotic syndrome increases cell death, up-regulates pro-apoptotic pathways and up-regulates inflammatory cytokines[27].***Ayesha and Peter*** examined the increase significant level of TGF-β in chronic kidney disease and how it interacted with the components of glomerular filtration barrier causing loss of function and proteinuria.[28]

Although elevated serum levels of cholesterol and triglycerides have been noted since the early descriptions of nephrotic syndrome, the long-term consequences of prolonged dyslipidaemia in NS remain relatively poorly understood [29].

Our study revealed a strong positive correlation between TGF-β and dyslipidemia which may assist in management the pathogenesis of NS. ***Nishida et al*** contributed the release of TGF- β in the messengial cells as inflammatory cytokine reflecting the cell proliferation occurred as a result of elevated levels of LDL, VLDL and triglycerides in nephrotic syndrome [30]. another laboratory study demonstrated that the overloaded cholesterol is taken into the renal tubule epithelial cells, causing suppression on cell proliferation, which may be the cause of kidney damage [31].

Our results are also in accordance with ***Mimi et al.*** trial demonstrating that LDL exerted a significant effect on the expression of CTGF and collagen IV in endothelial cells. They had shown that LDL increased TGF promoter activity, the mRNA and the protein levels of CTGF, TGF-β, and collagen IV in endothelial cells [32].

The relation between nephrotic syndrome, TGF-β and dyslipidemia is still far from fully understood and further studied are needed.

VI. Conclusion:

There is increasing evidence that TGF-β plays an important role in induction of proteinuria mainly albumin causing albuminemia along with subsequent glomerulosclerosis and renal interstitial fibrosis. Directly targeting TGF-β is an exciting approach to chronic renal disease. Our study revealed a significant negative correlation between serum levels of TGF-β and serum albumin. Despite our results demonstrated a strong positive correlation between TGF-β and dyslipidemia among nephrotic cases. Further understanding of the TGF-β pathways involved will lead to a range of novel therapies that will reduce proteinuria and prevent progressive decline in renal function.

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Table (1): Clinic-demographic characteristics, biochemical laboratory profiles in nephrotic patients and healthy controls

Parameters	Patient (N=50)	Control (N=50)	*p*-value
Age (Years)	7.4±1.1	7.36±1.0	0.9
Gender			
Male	28	28	0.99
Female	22	22	
Serum creatinine (mg/dl)	0.14±0.05	0.54±0.1	< 0.001*
Serum Urea (mg/dl)	8.94±1.2	8.59±1.12	0.21
Serum Albumin (mg/dl)	2.5±0.4	4.2±0.4	< 0.001*
Serum cholesterol (mg/dl)	404.3±55	126.1±2.3	< 0.001*
Serum Triglycerides (mg/dl)	463.4±93	120±17.8	< 0.001*
Serum LDL-C (mg/dl)	379.4±56	63.5±6.7	< 0.001*
Serum HDL-C (mg/dl)	29.7±5.4	62.6±6.6	< 0.001*
Serum TGF- β (pg/ml)	$160.49 \times 10^3 \pm 28.8$	$56.22 \times 10^3 \pm 25.9$	< 0.001*
Urinary protein (mg/dl)	1.45±0.2	0.17±0.07	< 0.001*

Quantitative data are represented as mean ± S.D.
(*) Statistically significant from control group (P < 0.05) b n /

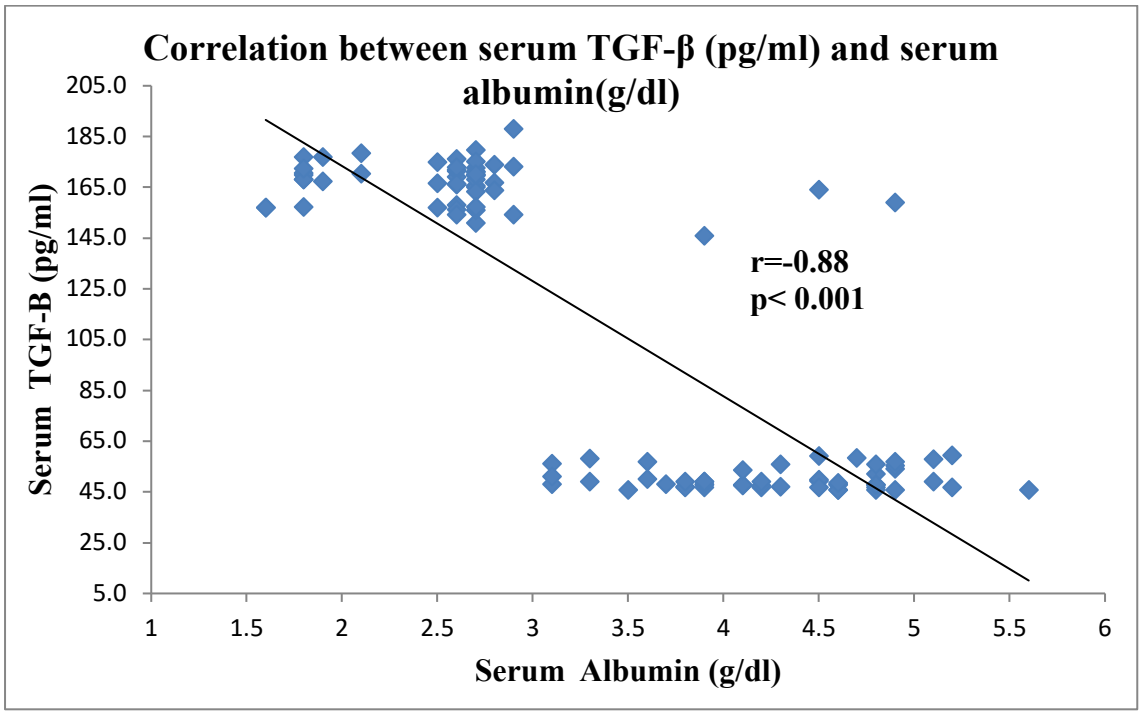

Figure (1): Correlation between Serum TGF-β and serum albumin

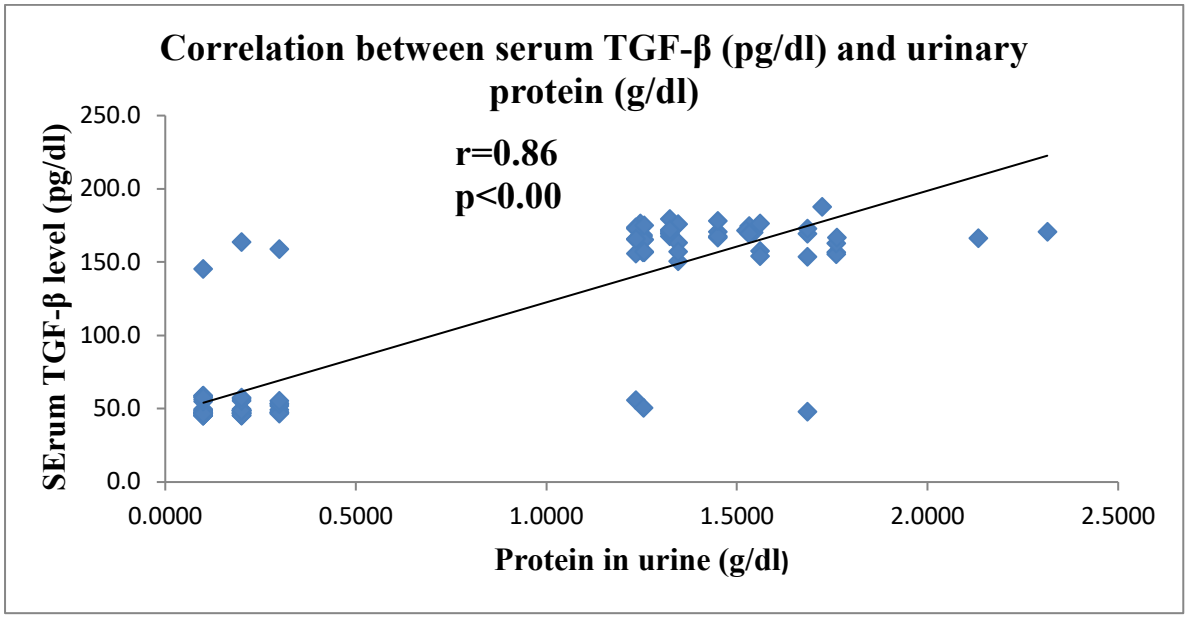

Figure (2): Correlation between Serum TGF-β and Urinary Total Protein

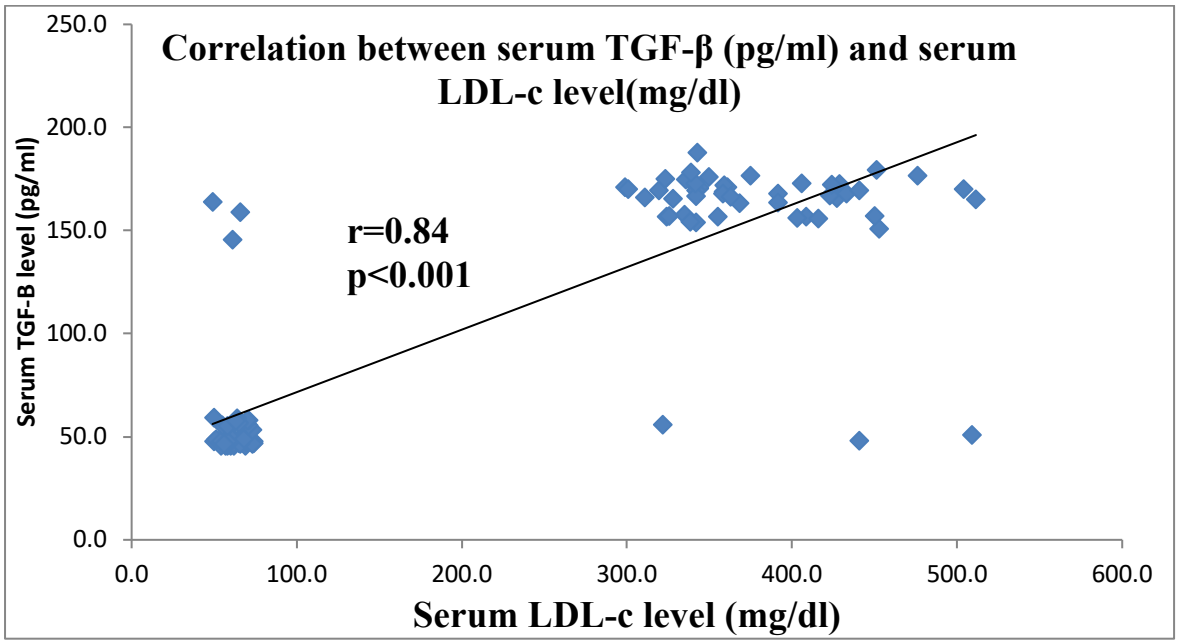

Figure (3): Correlation between Serum TGF-β and LDL-C

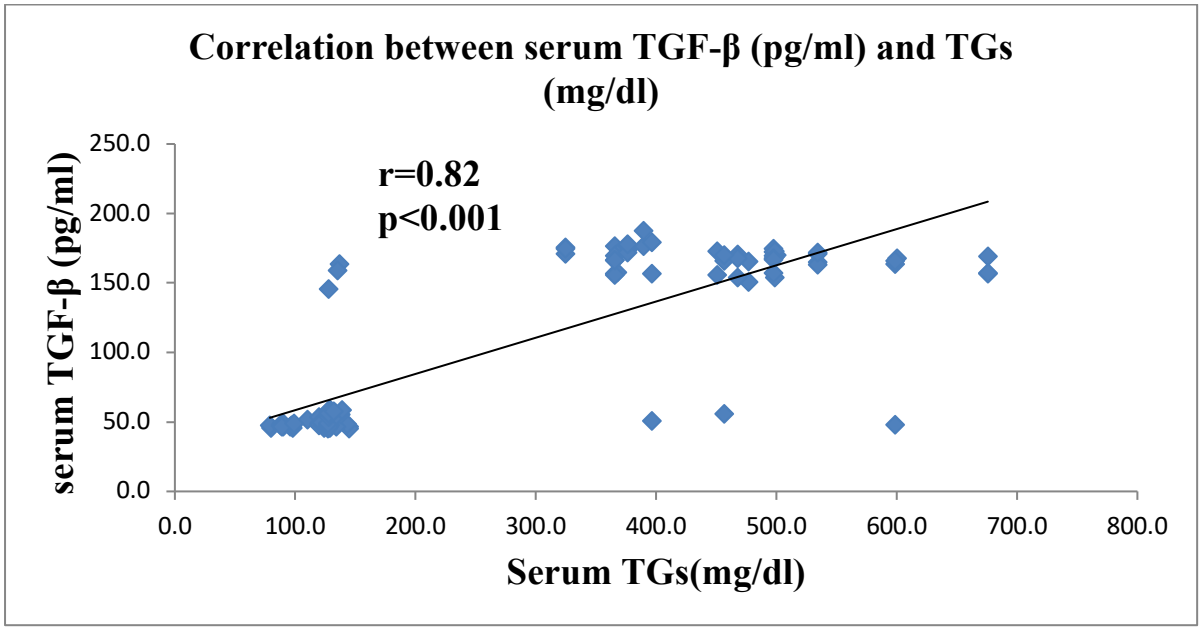

Figure (4): Correlation between Serum TGF-β and Triglycerides

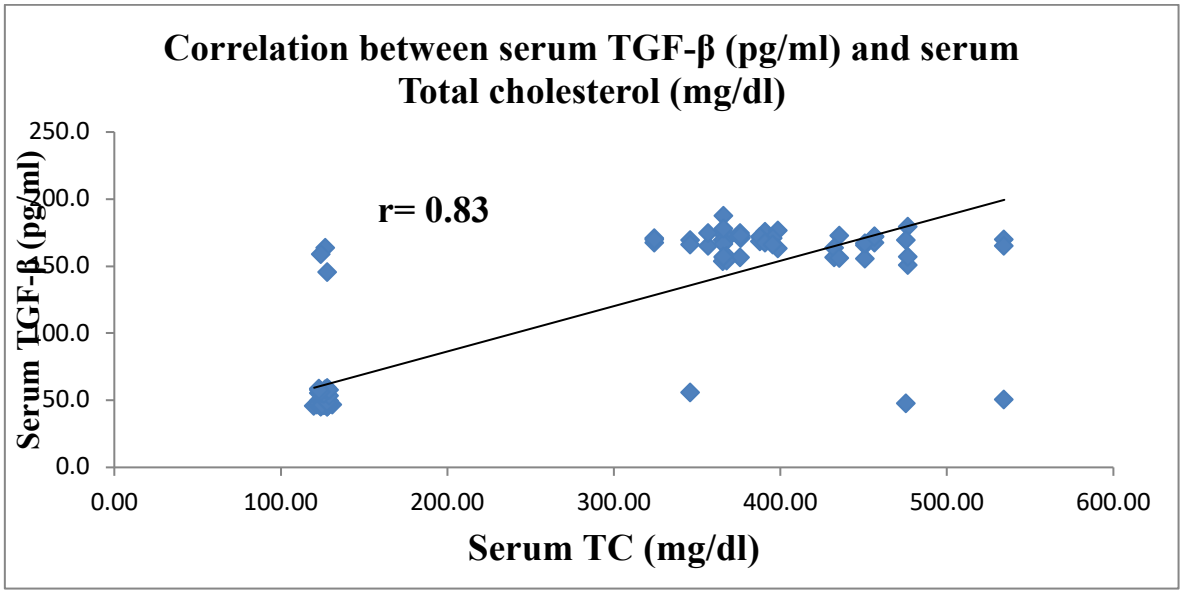

Figure (5): Correlation between Serum TGF-β and Total Cholesterol