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Assessment of Thrombospondin-1 Level in Type II Diabetic Patients.

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ABSTRACT

The study is intended to asses serum levels of Thrombospondin-1, fasting blood glucose in type 2 diabetic patients, also correlation between Thrombospondin-1 in both males and females. The study was conducted on randomly selected 65 type 2 diabetic patients (34 males and 31 females) attending the diabetes mellitus center in Al-Sadder Teaching City in Al- Najaf province , Iraqi and a group of 24 apparently healthy subjects (12 Males and 12 Females) were included as a control group . The Study was carried out from August 2013 to February 2014.The patients' age was ranging from 35 to 64 years old. The results indicated a significant increase ($p<0.05$) in serum FBG and TSP-1 levels in diabetic patients in comparing with healthy groups. The results also revealed no significant differences ($p> 0.05$) in serum TSP-1 levels between males and females in both patients and healthy groups, while the results of FBG levels significantly ($p<0.05$) increase in females than males in both patients and healthy groups. The results have been shown significant positive correlation ($P<0.05$) between TSP-1 and FBG in (males and females), males, females DM patients. The present study concluded that Thrombospondin-1 were marker for detection and diagnosis of diabetic patients type 2.

Keyword: Fasting blood glucose ,Thrombospondin-1, Type II diabetic patients.

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INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders of carbohydrates metabolism, this condition occurs when inadequate uptake of glucose by the cells of the body causes high levels of glucose in the blood (hyperglycemia) [1].The major forms of diabetes are divided into those caused by deficiency of insulin secretion due to pancreatic ß-cell damage (type 1 DM), and those that are a consequence of insulin resistance occurring at the level of skeletal muscle, liver, and adipose tissue, with various degrees of ß-cell impairment (type 2 DM) [2].

Type II diabetes is most commonly associated with the obesity in middle-aged individuals, it is due to reduction in the number or affinity of insulin receptors on the plasma membrane of cells in target tissues, or an abnormal binding of insulin to the receptors [3]. Patients with type I diabetes mellitus depend on external insulin (most commonly injected subcutaneously) for their survival because this hormone is no longer produced internally. Patients with type II diabetes mellitus are insulin resistant, have relatively low insulin production, or both, some patients with type II diabetes may eventually require insulin when other medications fail to control blood glucose levels adequately [4].

Thrombospondin-1 (TSP-1) is a well-studied glycoprotein that has four motifs (*i.e.*, adhesive domains; *N*-terminal domain, type I repeats, type III repeat and a *C*-terminal domain) [5] . TSP-1 suppresses endothelial cell proliferation, migration, and tube-formation and induces endothelial apoptosis [6;7;8] .Thus, TSP-1 is an endogenous inhibitor of angiogenesis under physiological and pathological conditions , including in the context of malignancy [9;10;11]. TSP-1 also modulates the extracellular matrix and leads to degradation and remodeling of connective tissues [12].

TSP-1 controls tissue perfusion and homeostasis by regulation of nitric oxide (NO) signaling [13]. Thus, the roles of TSP-1 in the regulation of angiogenesis are extremely complex and involve direct and indirect effects on stromal cells and the extracellular matrix. Regulation of angiogenesis and degradation of the extracellular matrix are also crucial steps for tumor growth, cancer cell invasion, and metastasis in nearly all solid tumors, and TSP-1 plays important roles for cancer cell invasion and dissemination in malignancies. In fact, the regulation of tumor growth and progression by TSP-1 [9]. TSP-1 plays an important role in the regulation of various biological activities, including vascular homeostasis, immunity, and wound healing [14;15;16].

MATERIALS AND METHODS

Patients and healthy groups

The study was performed on randomly selected 65 type 2 diabetic patients (34 males and 31 females) attending the diabetes mellitus center in Al-Sadder Teaching City Al- Najaf province , Iraqi . The patients' age was ranging from 35 to 64 years old. The study was carried out from the period August 2013 to February 2014.

Diabetes mellitus was diagnosed by consultant doctors. The information of patients were obtained through a questionnaire consisted of the name, sex, age, weight, height . Patients with renal dysfunction, heart diseases, who were on drugs affect oxidative stress, i.e., antioxidants, antihyperlipidemic agents were excluded from the current investigation.

A group of 24 apparently healthy subjects (12 Males and 12 Females) were included as a control group. Type 2 diabetic Patients are divided into subgroups according to the gender, ages, duration of disease and body mass index.

Collection of blood samples

Five milliliters of venous blood samples were drown using a disposable needle and plastic syringes from each patients and controls subject. Blood was left at room temperature for 10 minutes to clot, centrifuged 6000 rpm for 10 minutes, and then serum was separated and transported into new disposable tubes.

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Biochemical measurement

Determination of serum Thrombospondin-1 level

Thrombospondin ELISA Kit For the quantitative determination of human thrombospondin-1 (TSP-1) concentrations in serum was supplied by CUSABIO, USA.

Determination of fasting blood glucose concentration:

Glucose kit for quantitative determination of Glucose in human serum was supplied by Bio Merieux, France.

Statistical Analysis

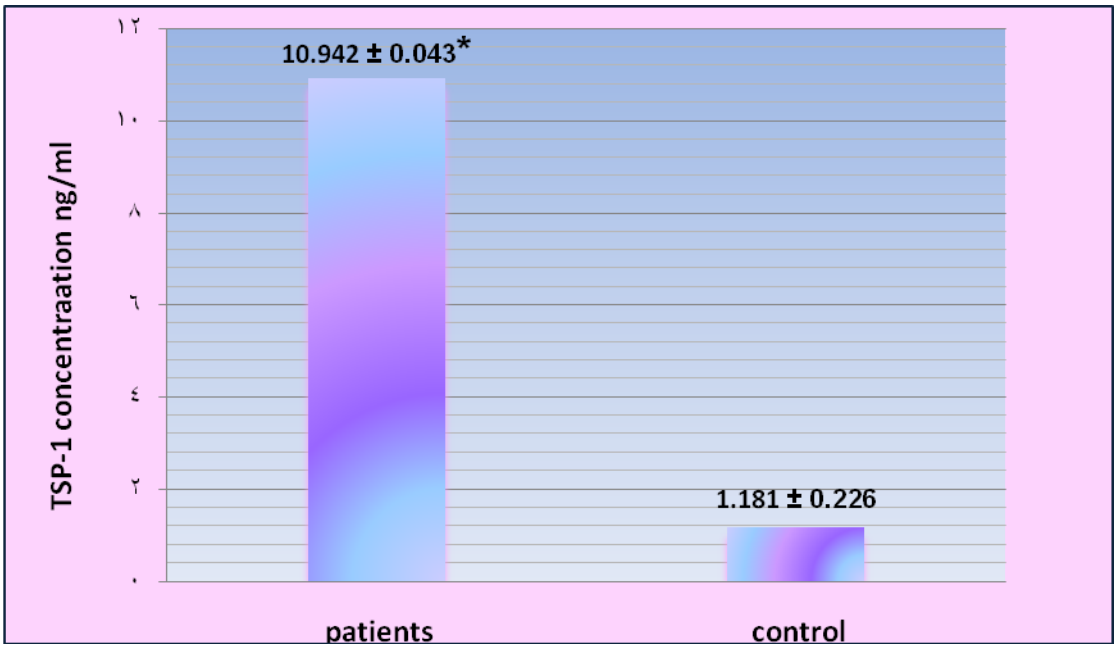
Analysis of data was performed by using megastat (Version 10.12) for excel 2007 in the home computer. The results were expressed as (mean ± standard deviation) .P-value < 0.05 was used as a level of statistically significant [17].

RESULTS

Comparison between diabetics mellitus patients and healthy groups

Thrombospondin-1 level

The results showed in figure (1)wereindicated a significant increase (P<0.05)inserumThrombospondin-1 (TSP-1) level in diabetic patients 10.942 ± 0.043 ng/ml in comparing the with control group 1.181 ± 0.226 ng/ml.



(*):Statistically significant differences (p<0.05) between patients and control groups.

Figure (1): Thrombospondin-1 level at diabetic mellitus patients and control groups.

Fasting blood glucose level

The results showed in figure (2) indicate a significant increase (P<0.05) in fasting blood glucose (FBG) level at diabetic patients 308.12 ± 9.10 mg/dl in comparing with healthy control group 116.38 ± 4.15mg/dl.

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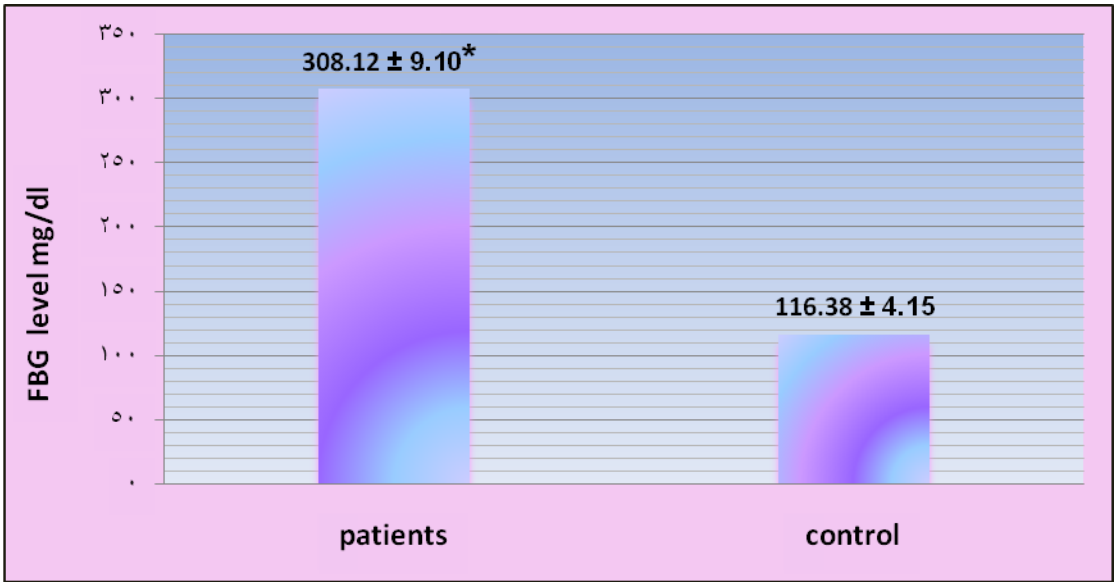

(*):Statistically significant differences at (p<0.05) between patients and control groups.

Figure (2): Fasting blood glucose level at diabetic mellitus patients and control groups.

Comparison between diabetic mellitus patients and healthy group according to gender

Thrombospondin-1 level

The results of table (1) reveal significant increase (p< 0.05) in serumthrombospondin-1 (TSP-1) level in both males and females patients groups in comparing with males and females of healthy groups respectively, while the table shows no significant differences (p>0.05) in serum TSP-1 level between males and females at both patients and healthy groups.

Table (1): Thrombospondin-1 level in both gender of diabetic mellitus patients and control groups.

Groups / Criteria	Mean ±S.E.			
	Control		Patients	
	n =24		n =65	
	Male	Female	Male	Female
	n = 12	n =12	n =34	n =31
TSP-1ng/ml	1.286 ± 0.069 [a]	1.077 ± 0.030 [a]	11.177± 0.284 [b]	10.684 ± 0.357 [b]

(The different letters refer to significant differences at level (P<0.05), same gender in different groups).(The same letters refer to no significant differences ,different gender in same groups).

Fasting blood glucose level

The results of table (2) reveal a significant increase (p< 0.05) in serum fasting blood glucose (FBG) level in females than males in both patients and healthy groups , while the table shows a significant increase (p<0.05) in serum FBG level at both males and females patients groups in comparing with males and females of healthy groups respectively.

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Table (2): Fasting blood glucose level in both gender of diabetic mellitus patients and control groups.

Groups / Criteria	Mean ±S.E.			
	Control		Patients	
	n =24		n =65	
	Male	Female	Male	Female
	n = 12	n =12	n =34	n =31
FBG mg/dl	96.25 ± 4.11[a]	119.50 ± 5.23 [b]	291.12 ± 11.32 [c]	326.77 ± 13.92 [d]

The different letters refer to significant differences at level (P<0.05) between groups.

Correlation between Thrombospondin-1 and Fasting blood glucose levels

The results of correlation and linear regression betweenTSP-1and FBG levels in patients are indicated: 1-The presence of a significant positive correlation at (P<0.05) between TSP-1and FBG concentrations of DM patients (males and females) , (r =0.978, TSP-1 =3.452+ 0.024 FBG) ,(figure 3).

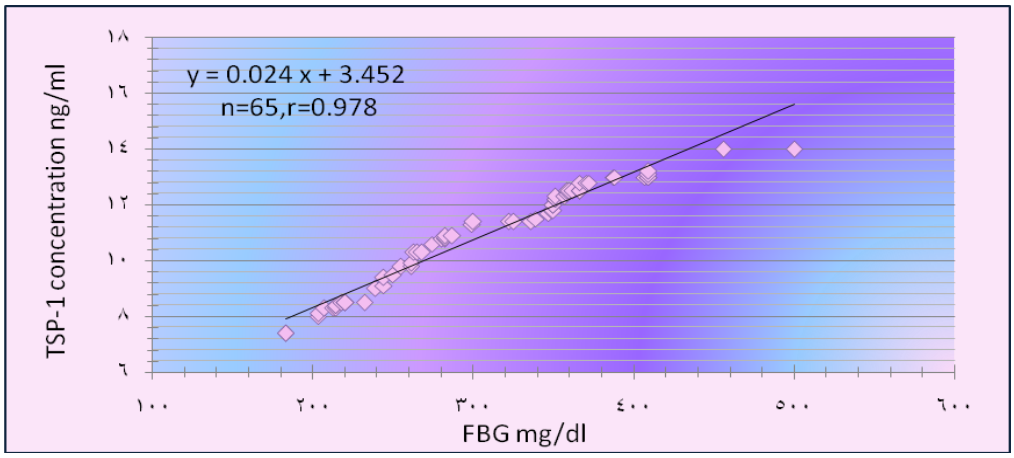

Figure (3):Correlation between Thrombospondin-1 and Fasting blood glucose in type 2 DM patients .

The presence of a significant positive correlation at (P<0.05) between TSP-1and FBG concentrations in females of DM patients, (r =0.976, TSP-1=2.504+ 0.025 FBG) , (figure 4).

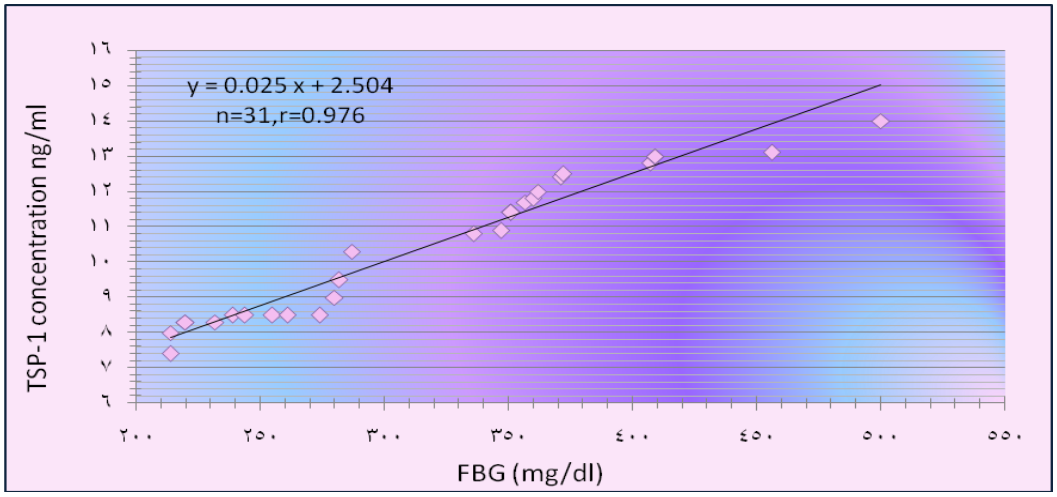

Figure (4):Correlation between Thrombospondin-1 and Fasting blood glucose in type 2 DM female patients.

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The presence of a significant positive correlation in males DM patients between TSP-1 and FBG concentrations at (P<0.05) , (r =0.969,TSP-1=4.087 + 0.024 FBG) , (figure 5).

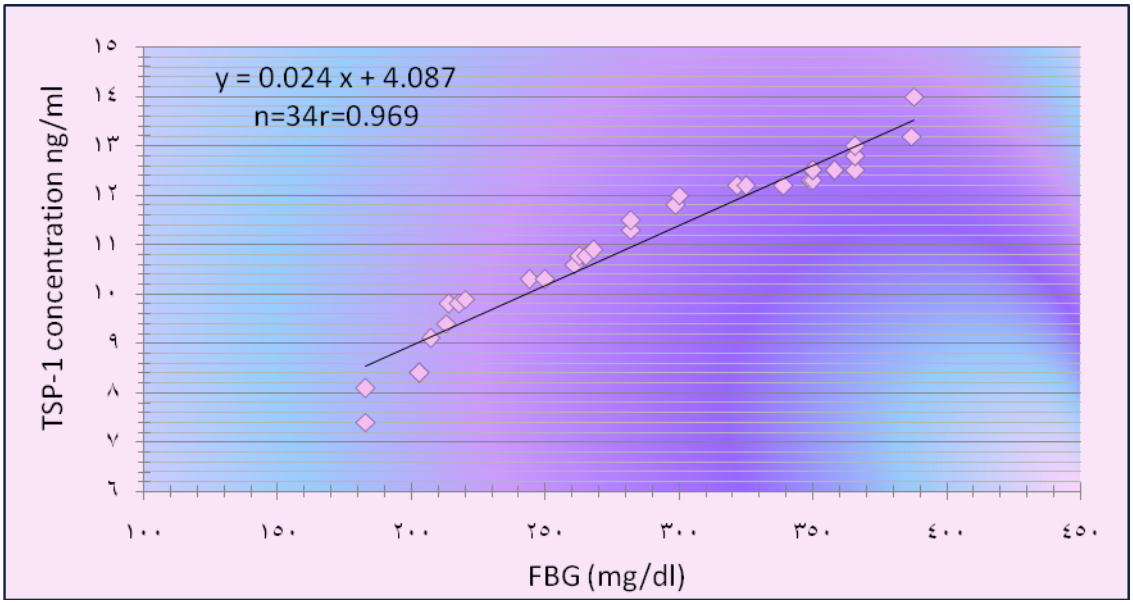

Figure (5):Correlation between Thrombospondin-1 and Fasting blood glucose in type 2 DM male patients.

DISCUSSION

Blood glucose level

The present results of presents study indicated significant increase (p<0.05) in serum blood glucose concentration in comparing with control group as presented in figure (2).

Most people of type 2 diabetic are still able to produce insulin at diagnosis, the insulin produced is unable to perform its primary job , which is helping the body cells used glucose for energy and this is due to a problem in the body insulin receptors also may be a problem with chemical made up of insulin itself and this conditions called insulin resistance [18;19;20].

The females patients showed significantly high levels of FBG than males as presented in table (2) , that result was agreement with other study [21]. Diabetes confers a markedly increased risk of cardiovascular events in both males and females [22]. However, women with diabetes are more susceptible to increased cardiovascular mortality [23].Diabetic women may be subject to more adverse changes in coagulation, vascular function and cardiovascular risk factors than diabetic men [24].

Thrombospondin-1 level

The present study revealed a significant increase (p<0.05) in Thrombospondin-1 level in diabetic patients in comparing with control groups as presented in figure (1).

Previous study suggested that expression of Thrombospondin-1 is markedly up regulated in tissue from diabetic patients and animal models [25;26]. Other researchers suggests that hyperglycemia potentially induced TSP-1 synthesis by vascular cells through pathways that may be involved glucose mediated activation of hexosamine [27;28].

Present studies agreement with other studies that's demonstrated relation between diabetes and TSP-1 [29;30].

Elevated blood glucose concentrations increased TSP-1 synthesis which was associated with reduced cell proliferation, increased TGF- β1 bioactivity and stimulation of fibronectin synthesis [31; 32]. TSP-1 loss also improved metabolic dysfunction , reduced blood glucose level [33].

Recent studies show that TSP-1 is highly expressed in various samples from various species including human and loss of TSP-1 in various diabetic rats [34;35].Under high glucose condition demonstrated decrease TSP-1 expression and down regulation of TSP-1 [34].

Previous study suggested that TSP-1 play an important roles in insulin resistance associated with type 2 diabetic and demonstrated that deletion or reduced TSP-1 may improved glucose-insulin homeostasis is associated with reduced inflammation and macrophage accumulation and decease adipose inflammation [36].In vitro studies support the effect of TSP-1 on macrophage mobility [37].

Voros *and* Lijnen.[38] found that obese TSP-1 deficient mice have improved glucose tolerance and insulin sensitivity. study of Winzer *et al*.[39] found of an association between pro inflammatory cytokines such tumor necrosis factor-α (TNF-α) and mRNA TSP-1 expression in subcutaneous adipose tissue.

Study of Belmadani *et al*.[40] showed that TSP-1 expression by cardiac fibroblast was up regulated in response to high glucose and also up regulated by mRNA and protein TGF-β1.

Study of Ramis *et al*.[41] confirmed that TSP-1 gene highly expressed in VAT females than males and that finding agreement with present study as presented in table (1). TSP-1 may be stimulated a proliferation of adipocyte and also effect adipocyte fatty acid in obese females than males [42; 43].

The present studies acceptance with other studies have reported that TSP-1 over expressed in obese human and rats [44;41] .TSP-1 is an adipokines which infiltrates adipose tissues during obesity [36].

The correlation between thrombospondin-1 and blood glucose

The results of correlation represent a positive significant correlation ($p<0.05$) between TSP-1 and blood glucose concentrations in both males and females , (male and female) diabetic patients as presented in figures (3,4, 5).

Previous studies have shown the hyperglycemia association with increase expression of TSP-1 [27] .Further studies showed that TSP-1 is expressed in the arterial wall of animal models of diabetic type 2, also in tissues from patients with diabetes [25; 45].

Many studies proved that TSP-1 may be important in the complications of diabetes mellitus by induced vascular smooth muscles (VSMCs) proliferation, migration and atherosclerosis in type 2 diabetes [46].

Few studies have examined the relation between TSP-1 and hyperglycemia by effect on VSMC gene expression. The combination of hyperglycemia and TSP-1 for 6 hour incubation period increased a number of gene altered, also hyperglycemia alone did not cause the down regulation of any gene [47;25] .

Previous work showed a relation between hyperglycemia and induces TSP-1 mRNA in VSMCs after 24 hours, which is consistent with our data [27].

Study of Maile *et al*.[48]showed that hyperglycemia mice was a significant increase ($p<0.05$) in TSP-1 and TSP-1 binding to CD47/IAP and this associated with enhanced proliferative response to insulin growth factor-1(IGF-1).

Previous studies suggest that significant contributor to the increase in TSP-1 protein due to its protection from lysosomal degradation, In addition to stability and rapid internalization by endocytic receptor LRP-1(low density lipoprotein-related protein) that leads to degradation in type 2 diabetes patients [49;50]. Further studies proved that the level of LRP-1 receptor differ from diabetes in comparing with normal glucose and these difference that account for the change in TSP-1 uptake [51]. Other studies focusing on certain markers and using other plant extract have been conducted by other researchers. Aldujali and colleagues [52]

showed that there is a significant Correlation between Cardiovascular Diseases in Obese Men with The Inflammatory Markers Dyslipidemia, C-Reactive Protein and Tumor Necrosis Factor-α. Further studies by Aldujali and colleagues [53,54,55] showed significant relationship Between Adipocyte Fatty Acid–Binding Protein In Obese Men With Cardiovascular Diseases and the Effect of Methanolic Leaf Extract of *Moringa oleiferaon* some Biochemical Markers in obesity induced rats. On the other hand they conducted a research between the effect of Methanolic Extract of *Moringa oleifera* and Exogenous Ghrelin on Lipid Profile in Atherogenic Rats and have found a significant impact of moringa extract on the reduction of lipid profiles in Atherogenic Rats [55].

CONCLUSION

Thrombospondin-1 is a good bio marker for prediction and diagnosis for type 2 diabetes mellitus and the study also proved a positive relation between thrombospondin-1 and blood glucose.

REFERENCES

[1] Canadian Diabetes Association (CDA). (2008). Clinical Practice Guideline for the Prevention and Management of Diabetes in Canada. *Canad. J. Diabet.*, 32(1):S1-S201.

[2] Alemzadeh, R. ;Wyatt, D.T.; Behrman, R.E.; Kliegman, R.M. and Jenson, H.B. (2008). Diabetes mellitus in children. *Nelson Textbook of Pediatrics.18th ed. Philadelphia: Saunders*., PP : 2404-31.

[3] Thabrew, I. and Ayling, R.M. (2001). Biochemistry for clinical Medicine. 1sted.*Green wich Medical Media ttd. London*. Pp.166-167.

[4] Leahy; Jack, L. and William, T. (2002). insulin Therapy.*1st ed.New York: Marcel Dekker. ISBN*.0-8247-0711-7.

[5] Qian, X.; Tuszynski, G.P.(1996). Expression of thrombospondin-1 in cancer: A role in tumor progression. *Proc. Soc. Exp. Biol. Med*., 212: 199–207.

[6] Taraboletti, G.; Roberts, D.D.; Liotta, L.A.; Giavazzi, R.(1990). Platelet thrombospondin modulates endothelial cell adhesion, motility, and growth: A potential angiogenesis regulatory factor.*J. Cell Biol*., 111: 765–772.

[7] Tolsma, S.S.; Stack, M.S.; Bouck, N.(1997). Lumen formation and other angiogenic activities of cultured capillary endothelial cells are inhibited by thorombospondin-1. *Microvasc. Res*., 54:13–26.

[8] Jimenez, B.; Volpert, O.V.; Crawford, S.E.; Febbraio, M.; Silverstein, R.L. and Bouck, N. (2000). Signals leading to apoptosis-dependent inhibition of neovascularization by thrombospondin-1. *Nat. Med.,* 6: 41-48.

[9] Lawler, J. (2002). Thrombospondin-1 as an endogenous inhibitor of angiogenesis and tumor growth. *J. Cell Mol. Med.,* 6:1-12.

[10] Armstrong, L.C.; Bormstein, P.(2003). Thrombospondins 1 and 2 function as inhibitors of angiogenesis. *Matrix Biol*., 22: 63–71.

[11] Adams, J.C., and Lawler, J. (2004). The thrombospondins. *Int. J. Biochem. Cell Biol*.,36:961-968.

[12] Lawler, J.(2000). The functions of TSP-1 and TSP-2. *Curr. Opin. Cell Biol*., 12: 634–650.

[13] Isenberg, J.S.; Martin-Manso, G.; Maxhimer, J.B.; Roberts, D.D. (2009).Regulation of nitric oxide signaling by thrombospondin-1: Implications for anti-angiogenic therapies. *Nat. Rev. Cancer*, 9: 182–194.

[14] Reed, M.J.; Puolakkainen, P.; Lane, T.F.; Dickerson, D.; Bornstein, P.; Sage, E.H.(1993). Different expression of SPARC and thrombospondin-1 in wound repair. *Immunolocalization and in situ hybridization. J. Histochem. Cytochem*., 41: 1467–1477.

[15] Bauer, E.M. ; Qin, Y.; Miller, T.W. ; Bandle, R.W. ; Csanyi, G. ; Pagano, P.J. ; Bauer, P.M. ; Schnermann, J. ; Roberts, D.D. ; Isenberg, J.S. (2010).Thrombospondin-1 supports blood pressure by limiting eNOS activation and endothelial-dependent vasorelaxation. *Cardiovasc. Res*., 88:471–481.

[16] Lopez-Dee, Z.; Pidcock, K.; Gutierrez, L.S. (2011). Thrombospondin-1: Multiple paths to inflammation. *Mediators Inflamm*.,1-10.

[17] Al-Rawi, K.(2000). Entrance to the Statistics. Second edition. *Faculty of Agriculture and Forestry, University of Mosul*.

[18] National Diabetes Information Clearinghouse (NDIC).(2008). National Diabetes Statistics.

[19] Ismail, B.H.(2010). Comparison Study of insulin level and lipid profile in diabetes mellitus in Ramadi city. *J. of university of Anbar for pure .sci*.,4(2):1-3.

[20] Paz ,V. ; Elena , L. and Marcia, H. (2006).Sexual dimorphism in insulin sensitivity and susceptibility to develop diabetes in rats. *J. End*., 190:425-432.

[21] Khan, H.A. (2006). Clinical significance of HbA1c as a marker of circulating lipids in male and female type 2 diabetic patients. *Acta. Diabet*.,1-8.

[22] Haffner, S.M.; Lehto, S.; Ronnemaa, T.; Pyorala, K.; Laakso, M. (1998). Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N. Engl. J. Med*., 339:229–234.

[23] Gu, K. ; Cowie, C.C. and Harris, M.I. (1999). Diabetes and decline in heart disease mortality in US adults. *JAMA*., 281:1291–1297.

[24] Steinberg, H.O.; Paradisi, G.; Cronin, J.; Crowde, K. ; Hempfling, A. ;Hook, G. and Baron, A.D. (2000). Type II diabetes abrogates sex differences in endothelial function in pre-menopausal women. *Circul.*, 101:2040–2046.

[25] Stenina,O.I.; Krukovets, I.; Wang, K.; Zhou, Z.; Forudi, F.; Penn, M.S. *et al*.(2003). Increased expression of thrombospondin-1 in vessel wall of diabetic Zucker rat. *Circul*, 107:3209-15.

[26] Kong, P.; Gonzalez-Quesada, C.; Li, N.; Cavalera, M.; Lee, D.W. and Frangogiannis, N.G.(2013a). Thrombospondin-1 regulates adiposity and metabolic dysfunction in dietinduced obesity enhancing adipose inflammation and stimulating adipocyte proliferation. *Am. J. Physiol. Endocrinol*. Metab., 305:E439-50.

[27] Raman, P.; Krukovets, I.; Marinic, T.E.; Bornstein, P. and Stenina, O.I.(2007).Glycosylation mediates up-regulation of a potent antiangiogenic and proatherogenic protein, thrombospondin-1, by glucose in vascular smooth muscle cells. *J. Biol. Chem*., 282:5704-14.

[28] Raman, P.; Harry, C.; Weber, M.; Krukovets, I. and Stenina, O.I.(2011). A novel transcriptional mechanism of cell type specific regulation of vascular gene expression by glucose. *Arterioscler. Thromb. Vasc. Biol*., 31: 634-42.

[29] Yung, S. ; Candice, Y.Y. ; Lee, Y. ; Zhang, L. Q. ; Lau, S.K. ; Tsang, R.C.W. and Chan, T.M. (2006). Elevated glucose induction of thrombospondin-1 up-regulates fibronectin synthesis in proximal renal tubular epithelial cells through TGF-b1 dependent and TGF-b1 independent pathways. *Nephrol. Dial. Transplant*, 21: 1504–1513.

[30] Tang, M. ; Zhou, F. ; Zhang, W. ; Guo, Z. ;Shang Y. ; Lu, H. ; Lu, R. ; Zhang, Y. ; Chen, Y. and Zhong, M. (2011).The role of thrombospondin1-mediated TGF-b1 on collagen type III synthesis induced by high glucose. *Mol.Cell. Biochem*., 346:49–56.

[31] Murphy-Ullrich, J.E. and Poczatek, M.(2000). Activation of latent TGF-beta by thrombospondin1: mechanisms and physiology. *Cytok. Grow. Fact. Rev.,* 11: 59–69.

[32] Yevdokimova, N. ; Wahab, N.A. and Mason, R.M.(2001). Thrombospondin-1 is the key activator of TGF-b1 in human mesangial cells exposed to high glucose. *J. Am. Soc. Nephrol*., 12: 703–712.

[33] Kong, P. ; Cavalera, M. and Frangogiannis, N.G.(2013b). The role of thrombospondin-1 (TSP-1) in obesity and diabetes. *Adipocyte* , 3:1:1-4.

[34] Sheibani, N.; Sorenson, C.M.; Cornelius, L.A. and Frazier, W.A. (2000). Thrombospondin-1, a natural inhibitor of angiogenesis, is present in vitreous and aqueous humor and is modulated by hyperglycemia. *Biochem. Biophys. Res. Commun*., 267: 257-261.

[35] Wang, S.; Gottlieb, J.L. ; Sorenson, C.M. and Sheibani, N. (2009). Modulation of thrombospondin-1 and pigment epithelium-derived factor levels in vitreous fluid of patients with diabetes. *Arch. Ophthalmol.*, 127: 507-513.

[36] Varma, V.; Yao-Borengasser, A.; Bodles, A.M.; Rasouli, N.; Phanavanh, B. *et al*.(2008). Thrombospondin-1 is an adipokine associated with obesity, adipose inflammation, and insulin resistance. *Diabet.*, 57: 432–439.

[37] Li ,Y. ; Tong, X. ; Rumala, C. ; Clemons, K. and Wang, S. (2011). Thrombospondin1 deficiency reduces obesity-associated inflammation and improves insulin sensitivity in a diet-induced obese mouse model. *PLoS. One*. , 6: e26656.

[38] Voros, G. and Lijnen, H.R. (2006). Deficiency of thrombospondin-1 in mice does not affect adipose tissue development. *J. Thromb*. Haemost., 4: 277–278.

[39] Winzer, C. ;Wagner, O.; Festa, A. *et al*.(2004). Plasma adiponectin, insulin sensitivity, and subclinical inflammation in women with prior gestational diabetes mellitus. *Diabet. Care*, 27: 1721-7.

[40] Belmadani, S.; Bernal, J.; Wei, C.C.; Pallero, M.A.; Dell'italia, L.; Murphy-Ullrich, J.E. and Berecek, K.H. (2007). A thrombospondin-1antagonist of transforming growth factor-beta activation blockscardiomyopathy in rats with diabetes and elevated angiotensin II.*Am. J. Pathol*., 171(3):777–789.

[41] Ramis, J.M.;Franssen-van Hal, N.L.; Kramer, E.; Llado, I.; Bouillaud, F. *et al*. (2002).Carboxypeptidase E and thrombospondin-1 are differently expressed in subcutaneous and visceral fat of obese subjects. *Cell. Mol. Life Sci*., 59: 1960- 1971.

[42] Hajri , T.; Han ,X.X.; Bonen, A. and Abumrad, N.A.(2002). Defective fatty acid uptake modulates insulin responsiveness and metabolic responses to diet in CD36-null mice. *J. Clin. Invest*., 109:1381-9.

[43] Hajri, T.; Hall, A.M.; Jensen, D.R.; Pietka, T.A.; Drover, V.A.; Tao, H.; Eckel, R. and Abumrad, N.A.(2007). CD36-facilitated fatty acid uptake inhibits leptin production and signaling in adipose tissue. *Diabet.*, 56: 1872-80.

[44] Hida, K.; Wada, J.; Zhang, H.; Hiragushi, K.; Tsuchiyama, Y.; Shikata, K. and Makino, H.(2000). Identification of genes specifically expressed in the accumulated visceral adipose tissue of OLETF rats. *J. Lipid Res.,* 41:1615–1622.

[45] Wahab, N.A.; Schaefer, L.; Weston, B.S. *et al*.(2005).Glomerular expression of thrombospondin-1, transforming growth factor beta and connective tissue growth factor at different stages of diabetic nephropathy and their interdependent roles in mesangial response to diabetic stimuli. *Diabet.,* 48: 2650–2660.

[46] Maier, G.M. ; HAN, X. ; Sadowitz, B.; Gentile, K.L.; Middleton, F.A. and Gahton, V. (2009). Thrombospondin-1: A proatherosclerotic protein augmented by hyperglycemia. *J. Vas. Surg.*, 51(5):1238-1247.

[47] Adams, J.C. (1997). Thrombospondin-1. *Int. J. Biochem. Cell. Biol*., 29: 861-865.

[48] Maile, L. A.; Capps, B. E.; Miller, E. C. ; Aday, A. W. and Clemmons, D. R. (2008). "Integrin-associated protein association with src homology 2 domain containing tyrosine phosphatase substrate 1 regulates IGF-i signaling in vivo," *Diabet* ., 57(10): 2637–2643.

[49] Chen, H.; Sottile, J. ; Strickland, D. K. and Mosher, D. F. (1996a). Binding and degradation of thrombospondin-1 mediated through heparan sulphate proteoglycans and low-densitylipoprotein receptor-related protein: localization of the functional activity to the trimeric N-terminal heparin-binding region of thrombospondin-1. *Biochem. J.,* 318(3): 959–963.

[50] Chen, H. ; Strickland, D. K. and Mosher, D. F.(1996b). Metabolism of thrombospondin-2: binding and degradation by 3T3 cells and glycosaminoglycan-variant Chinese hamster ovary cells. *J . Biol. Chem.*, 271 (27):15993– 15999.

[51] Hong, H. ; Liu, L. P. ; Liao, J. M. et al.(2009). Downregulation of LPR1 at the blood-brain barrier in streptozotocin-induced diabetic mice. *Neuropharmacol* ., 56(6-7) 1054–1059.

[52] H, Hammod, A, Al-Dujaili, and M, Al-Dujaili.(2016). The Correlation between Cardiovascular Diseases in Obese Men with The Inflammatory Markers: Dyslipidemia, C-Reactive Protein and Tumor Necrosis Factor-α. RJPBCS 7(3) Page No. 809.

[53] H, Hammod, A, Al-Dujaili, and M, Al-Dujaili.(2016). Relationship Between Adipocyte Fatty Acid–Binding Protein In Obese Men With Cardiovascular Diseases. RJPBCS 7(3) Page No. 809.

[54] H, Saleem, A, Al-Dujaily2*, M, Al-murshidi. (2016). Effect of Methanolic Leaf Extract of *Moringa oleiferaon* some Biochemical Markers in obesity induced rats. RJPBCS 7(3) Page No. 809.

[55] H, Hammod, A, Al-Dujaili, and M, Al-Dujaili.(2016). Comparison Study between effect of Methanolic Extract of *Moringa oleifera* and Exogenous Ghrelin on Lipid Profile in Atherogenic Rats. RJPBCS 7(3) Page No. 809.