Estimation Levels of Osteoponetin, Glutathione Peroxidase and Vitamin D3 in Type II Diabetic Patients with Renal Failure Undergoing Hemodialysis

Rasha Hasan Jasim and Wedyan Mohammed Torki

Department of Chemistry, Faculty of Education for Girls, University of Kufa, Iraq

e-mail: ***dr.rashahussainee@yahoo.com***

ABSTRACT

Backgrounds: ***Twenty to twenty five percentage of T2DM patients eventually develop nephropathy depending on genetic predisposition. It is the cause of death of 17% of these patients. The exact mechanisms which cause diabetic nephropathy are unknown, although probably multifactorial, the cause of nephropathy may be due to the accumulation of a reduced sugar product (sorbitol) or a tissue toxin that can affect the Na-K-ATP pump. However, in T2DM, mesangiallymphokine production is associated not only with hyperglycemia but also with insulin resistance and generalized vascular disease, thus albuminuria may occur even before hyperglycemia develops.*** ***Subjects:*** ***The current study included 88 people who were divided into two groups: the first included 50 patients suffering from kidney failure (18 females and 32 males), their ages range between (36-78 years) and they are subject to dialysis, while the second group included 38 individuals (11 females and 27 males) their ages range between (31-64 years), as a control group.*** ***Aim of The Study:*** ***The current study is designed to find out the levels of Osteoponetin, Glutathione Peroxidase, 1,25 Di hydroxyl vitamin D3 in the sera of study individuals.*** ***Conclusions:*** ***Kidney complications of type II diabetes can develop in the patients regardless their gender or age. Osteoponetin is an excellent diagnostic tool for diagnosing kidney failure caused by the complications of diabetes and for predicting the kidney's efficiency to perform its vital functions. The capacity of cellular defense enzymes diminishes with increasing cellular oxidative stress as the renal damage from complications of type II diabetes reaches the stage of renal failure requiring dialysis. Vitamin D levels are considered low among the people studied, whether they are diabetics undergoing dialysis or in good health.***

KEY WORDS: Osteoponetin, Glutathione Peroxidase and Vitamin D3, Type IIDM, Renal Failure

INTRODUCTION

Type II Diabetes Mellitus (T2DM) is characterized by insulin resistance at the level of skeletal muscle and fat tissue and muscle and liver, which leads to an increase in the level of glucose in the blood. This type of diabetes is not dependent on insulin, which affects adults or diabetes resistant ketosis, when the pancreas continues to produce insulin, but there is an imbalance in insulin receptor cells that does not respond to insulin, causing improper hepatic

glucose metabolism [1]. Diabetic nephropathy is a kidney disorder that is a complication of diabetes mellitus, and it is characterized by proteinuria and progressive reduction in kidney function culminating in azotemia. Common names of this disorder are Kimmelstiel-Wilson disease, diabetic glomerulosclerosis, and diabetic kidney disease [2]. Kidney damage caused by diabetes most often involves thickening and hardening (sclerosis) of the internal kidney structures, particularly the glomerulus. Diabetic nephropathy can sometimes cause nephritic syndrome, which may lead to acute renal failure. The disorder continues to progress rapidly with the appearance of macroproteinuria, with end-stage renal disease (ESRD)[3] typically developing within 2-6 years after the appearance of chronic renal failure or significant hypertension, and within 10 years after diagnosis of diabetes [4].

Osteoponetin (OPN) is a combination of the words "osteo" meaning "bone" and "pontin" meaning "bridge", is a highly phosphorylated and glycosylated sialoprotein consisting of approximately 314 amino acids with a molecular weight ranging between 44 and 75 k Da [5,6], that is expressed by several cell types including osteoblasts, and osteoclasts [7] epithelial and mesenchymal cells and cells of hematopoietic origin like T cells and macrophages [8]. OPN belongs to the family of non-collagenous [9]. OPN can be considered an extracellular matrix protein, it was identified in 1985 by Heingard [10,11]. OPN molecule comprises unique conserved regions which involve (RG)-binding domain, serine/threonine phosphorylation site, two heparin-binding sites, one thrombin cleavage site, and a putative calcium-binding site, the cell interacting domains include arginine-glycine-aspartic acid (RGD) cell-binding sequence and serine-valine-valine-tyrosine-glutamate-leucine-arginine [9]. High OPN expression is detected in bone, joints, adipose tissue, liver, lung, brain, and body fluids including blood, urine, bile, and milk [12]. It is also found in kidneys (in the thick ascending limbs of the loop of Henley and in distal nephrons [13]. It has important roles in health and it is a multifunctional protein which has important functions on cardiovascular diseases, cancer, diabetes and kidney stone diseases [5]. The protein does not only play an important role in mineralization and bone resorption, but also acts as a regulator of immune response [14]. It was demonstrated that OPN regulates migration and infiltration of macrophages [13]. It believed to exacerbate inflammation in several chronic inflammatory diseases [15].

Glutathione peroxidase (GPx) is the general name of an enzyme family with peroxidase activity, is an antioxidant enzyme class with the capacity to scavenge free radicals. This is in turn helps to prevent lipid peroxidation and maintain intracellular homeostasis as well as redox balance [16]. Most times, its activity depends on a micronutrient cofactor known as

selenium for this reason, GPX is often referred to as a selenocysteine peroxidase. The antioxidant defense system of the body depends on large and various protection mechanisms to keep ROS originating from endogenous or exogenous sources at physiologically optimal levels. The enzyme plays a more crucial role of inhibiting lipid peroxidation process, and therefore protects cells from oxidative stress [8]. It plays an important role in the maintenance of the reactive oxygen species (ROS) metabolic balance *in vivo*[17]. In the absence of this antioxidant enzyme, a buildup of ROS ensues that is known to damage DNA, proteins, and lipids[18]. GPx1 is the most abundant selenoperoxidase and is present virtually in all cells. GPx2 is found much more in the gastrointestinal tract primarily in the intestine The kidney relative to other tissues is the primary location for GPx3,though; the enzyme is also present in extracellular fluids as a glycoprotein.

Vitamin D was initially described as a substance capable of treating rickets and was called "D" because it is the fourth in the sequence of vitamins discovered. Vitamin D is a lipid-soluble vitamin, and also involved in cellular differentiation and regeneration of various organs[19]. Vitamin D has a crucial role in regulating the metabolism of calcium and phosphate through its effects on the intestines, bones, and kidneys. It is particularly important for optimal intestinal calcium absorption and exerts significant effects on bones by both maintaining mineral balance and directing multiple effects on bone cell[20].

Vitamin D3 chemically (1α,25-dihydroxycholecalciferol) is an organic steroid substance, historically classified as a vitamin, in fact it is a hormonal compound. At the basal layer of epidermis, the cholesterol is converted to pro-vitamin D3 (7-dehydrocholesterol), which undergoes a photochemical transformation due to ultraviolet, then a slow isomerization process under the influence of temperature results in a production of cholecalciferol (traditionally called vitamin D3).The first activation is made in hepatic cells into 25-hydroxycholecalciferol [25(OH)D] (calcidiol),which is the main form of vitamin circulating in the blood. The second stage occurs in the kidney cells with the production of 1α,25-dihydroxycholecalciferol [1,25(OH)D] (calcitriol), which is the main active form of the vitamin [21].

MATERIAL AND METHOD

During the extended period from the beginning of August 2019 to the end of January 2020, 88 residents of Al-Najaf Al-Ashraf Governorate were enrolled to participate in thecurrent study**.** Fifty diabetic patients with renal failure were included in the present work,

initial diagnosis of the injury type was completely performed by specialist physicians in Al-Najaf Al-Ashraf Governorateand throughseveral of clinical and laboratory tests. According to the questionnaire approved in the current work and designed according to the opinion of specialists,which includes information on the following: age, gender,place of residence, the duration of the first symptoms ofthe diseases, other diseases experienced by patients,treatments used by the patients, and family history. Full

information was provided on the patients of the present study through oral interviews with patients and incooperation with the supervising physicians. Selection of healthy individuals as a control group (38 individuals) based on several criteria; included: nonsmokers, no medical history of any gastrointestinal orurinary disorder, individuals should not take any medication and have not undergone surgical through lastyears, and a subjective perception of good health asdetermined by health questionnaire. More than, controlgroup might at approximate age range with the patient'sgroup, no alcohol drinking, with similar food style to patients' group**.** After obtaining the required official approvals andrecording the current study in the annual plan of the Ministry of Health, samples of renal failure were collectedfrom patients registered for treatment at the center of kidney disease and transplantation AL-Sadder dialysis center in Al-Sadder Medical City in Al-Najaf after completing the clinical diagnosis process by specialistdoctors and during dialysis.While the other infectedsamples were collected from Al-Hakeem Hospital in Al-Najaf Al-Ashraf Governorate. The control group samples were collected from theexperimental environment after ensuring the adequacy ofthe criteria specified in this study.

Concentration of serum Osteoponetin and Glutathione Peroxidase were measured using Sandwich ELISA while concentration of serum vitamin D3 was measured using competitive ELISA.

RESULTS AND DISCUSSION

Osteoponetin and glutathione peroxidase revealed statistical significance of these proteins when comparing healthy and patients groups together, while vitamin D3 show no significant difference when comparing the levels of this vitamin in the two main study groups (**Table 1**).

Table 1: Levels (Mean ± S.D.) of Osteoponetin (ng/mL), Glutathione Peroxidase (pg/mL) and Vitamin D3 (pg/mL) Concentration in the Sera of Diabetic Patients and Controls Subjects

Parameters	***Diabetic Patients***	***Healthy Controls***	***p-value***
	Mean ± S.D.	***Mean ± S.D.***	
Osteoponetin	9.003±0.411	11.228±1.135	0.050

(ng/mL)			
Glutathione Peroxidase (pg/mL)	558.231±30.311	601.066±46.621	0.047
Vitamin D3 (pg/mL)	81.238±9.006	96.836±9.984	0.071

The mean difference is significant at the 0.05 level

The statistical analysis of the present results lacked significance when performing the implicit comparison between sexes in both major groups (patients and control groups) where no significant differences were observed when performing the comparison by the ANOVA test between women and men in the same group (**p = 0.311** in the patients group) (**p = 0.063** in the control group).

When comparing the males of the two studied groups together, the results show that there was a significant variation **(p=0.046)** resulting from the low level of osteoponetin in the sera of the group of patient males with renal failure due to the complications of type 2 diabetes and subject to dialysis versus males in the control group. On the other hand, the results were contradictory to those results obtained when comparing the levels of this criterion between females in the two groups studied**(P=0.056)**.

While when the Glutathione Peroxidase was determined, the Statistical analysis of the data by ANOVA demonstrated that only statistically significant difference was recorded when comparing females with renal failure caused by the complications of type 2 diabetes and healthy females in the current study**(P=0.005)**, while the two subgroups of males failed to record similar results when making the same comparison**(P=0.528)**.

Additionally, the statistical significance was absent from the differences between the sexes within the same group, whether among patients or healthy subjects.

The lowest levels of 1, 25 Dihydroxy Vitamin D3 (34.890 pg/mL) was recorded in the case of male patient aged 36 years old, while the highest concentration of this vitamin (154.014 pg/mL) was found in the sera sample of the youngest healthy male (31 years old).

The statistical analysis of the 1, 25 Dihydroxy Vitamin D3 data of the four subgroups by ANOVA test established that the statistical significance was absent from the differences between the sexes within the same group, whether among patients or healthy subjects.

Furthermore, only the statistically significant difference was recorded when comparing males with renal failure caused by the complications of type 2 diabetes and healthy males in

the present study, while the two subgroups of females failed to record similar results when making the same comparison.

Table 2 shows significant positive relationships when combined Osteoponetin to vitamin D3 and Glutathione Peroxidase, respectively; in the diabetic group, as well as when Glutathione Peroxidase and vitamin D3 were associated together in both of the study groups. Om the other hand, the relationships between Osteoponetin and vitamin D3 in addition to Glutathione Peroxidase appeared negative in the healthy group.

Table 2: Correlation Among The Evaluated Parameters in The Two Study Group

Subjects	*Diabetic Patients*	*Healthy Controls*
Osteoponetin *vs* Glutathione Peroxidase	$r = 0.782$ $p < 0.001$	$r = -0.843$ $p = 0.000$
Osteoponetin *vs* Vitamin D3	$r = 0.789$ $p < 0.001$	$r = -0.105$ $p > 0.05$
Glutathione Peroxidase *vs* Vitamin D3	$r = 0.698$ $p < 0.005$	$r = 0.474$ $p = 0.05$

The mean difference is significant at the 0.05 level

Diabetes mellitus is associated with the decrease of the antioxidant capacity and the increased production of ROS by increasing the proteins and oxidizing the DNA products and lipids [22,23]. Moreover, vitamin D deficiency is a risk for type I and Π diabetes. The stress caused by the ROS plays a critical role in the DM disease occurring as well as their complication progression, through insulin resistance or insulin secretion[24,25,26].

Glutathione peroxidase is considered a champion in detoxifying fat peroxide by converting hydrogen peroxide into fatty alcohol and water. Recent studies have confirmed a positive relationship between vitamin D3 and glutathione peroxidase [22].

A previous study has shown that Vitamin D has an antioxidant property in diabetes[27], oxidative stress is caused by both an increased formation of plasma free radicals and a reduction in antioxidant defenses. The decreased insulin and a high level of glucose sugar causes free radical formation and thus oxidative stress is produced[28].In the stages of type Π diabetes, the antioxidant defense system opposes free radicals, this means that antioxidants have an increased enzyme activity as a compensatory response to oxidative stress[29].

CONCLUSIONS

Kidney complications of type Π diabetes can develop in the patients regardless their gender or age. Osteoponetin is an excellent diagnostic tool for diagnosing kidney failure caused by the complications of diabetes and for predicting the kidney's efficiency to perform its vital functions. The capacity of cellular defense enzymes diminishes with increasing cellular oxidative stress as the renal damage from complications of type Π diabetes reaches the stage

of renal failure requiring dialysis. Vitamin D levels are considered low among the people studied, whether they are diabetics undergoing dialysis or in good health.

REFERENCES

[1]K. Blaslov, F. S. Naranđa, I. Kruljac, and I. P. Renar, "**Treatment approach to type 2 diabetes: Past, present and future**," *World J. Diabetes*, vol. 9, no. 12, pp. 209–219, 2018, doi: 10.4239/wjd.v9.i12.209.

[2]K. Umanath and J. B. Lewis, "**Update on Diabetic Nephropathy: Core Curriculum 2018**," *Am. J. Kidney Dis.*, vol. 71, no. 6, pp. 884–895, 2018, doi: 10.1053/j.ajkd.2017.10.026.

[3]D. J. Den Hartogh and E. Tsiani, ***Health benefits of resveratrol in kidney disease: Evidence from in vitro and in vivo studies***, vol. 11, no. 7. 2019.

[4]K. C. Keri, N. S. Samji, and S. Blumenthal, "**Diabetic nephropathy: newer therapeutic perspectives**," *J. Community Hosp. Intern. Med. Perspect.*, vol. 8, no. 4, pp. 200–207, 2018, doi: 10.1080/20009666.2018.1500423.

[5]M. A. Icer and M. Gezmen-Karadag, "**The multiple functions and mechanisms of osteopontin**," *Clin. Biochem.*, vol. 59, no. September,pp. 17–24, 2018, doi: 10.1016/j.clinbiochem.2018.07.003.

[6]I. Abdelaziz Mohamed, A.-P. Gadeau, A. Hasan, N. Abdulrahman, and F. Mraiche, "**Osteopontin: A Promising Therapeutic Target in Cardiac Fibrosis**," *Cells*, vol. 8, no. 12, p. 1558, 2019, doi: 10.3390/cells8121558.

[7]X. Gui *et al.*, "**Prognostic Value of Serum Osteopontin in Acute Exacerbation of Idiopathic Pulmonary Fibrosis**," *Biomed Res. Int.*, vol. 2020, 2020, doi: 10.1155/2020/3424208.

[8]O. M. Ighodaro and O. A. Akinloye, "**First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid**," *Alexandria J. Med.*, vol. 54, no. 4, pp. 287–293, 2018, doi: 10.1016/j.ajme.2017.09.001.

[9]A. Singh, G. Gill, H. Kaur, M. Amhmed, and H. Jakhu, "**Role of osteopontin in bone remodeling and orthodontic tooth movement: a review**," *Prog. Orthod.*, vol. 19, no. 1, 2018, doi: 10.1186/s40510-018-0216-2.

[10]T. Iida, K. Wagatsuma, D. Hirayama, and H. Nakase, "**Is osteopontin a friend or foe of cell apoptosis in inflammatory gastrointestinal and liver diseases?**," *Int. J. Mol. Sci.*, vol. 19, no. 1, 2018, doi: 10.3390/ijms19010007.

[11]H. Nakase, "**OPeNing the Epithelial Barrier: Osteopontin Preserves Gut Barrier Function During Intestinal Inflammation**," *Dig. Dis. Sci.*, vol. 64, no. 2, pp. 294–296, 2019, doi: 10.1007/s10620-018-5350-7.

[12]M. R. Shurin, "**Osteopontin controls immunosuppression in the tumor microenvironment**," *J. Clin. Invest.*, vol. 128, no. 12, pp. 5209–5212, 2018, doi: 10.1172/JCI124918.

[13]A. Nephropathy, "**Excretion in Patients with Immunoglobulin**," pp. 1–10, 2019.

[14]N. Nourkami-Tutdibi, N. Graf, R. Beier, M. Zemlin, and E. Tutdibi, "**Plasma levels of osteopontin from birth to adulthood**," *Pediatr. Blood Cancer*, no. February, p. e28272, 2020, doi: 10.1002/pbc.28272.

[15]R. Bruha, L. Vitek, and V. Smid, "**Osteopontin – A potential biomarker of advanced liver disease**," *Ann. Hepatol.*, 2020, doi: 10.1016/j.aohep.2020.01.001.

[16]B. H. Xiao *et al.*, "**Corrigendum to 'Glutathione Peroxidase Level in Patients with Vitiligo: A Meta-Analysis**,'" *Biomed Res. Int.*, vol. 2017, no. December 2015, p. 6707162, 2017, doi: 10.1155/2017/6707162.

[17]J. A. de Oliveira-Silva *et al.*, "**Oxidative stress assessment by glutathione peroxidase activity and glutathione levels in response to seleniumsupplementation in patients with mucopolysaccharidosis I, II and VI**," *Genet. Mol. Biol.*, vol. 42, no. 1, pp. 1–8, 2019, doi: 10.1590/1678-4685-GMB-2017-0334.

[18]M. Maciejczyk, A. Zalewska, and J. R. Ładny, "**Salivary Antioxidant Barrier, Redox Status, and Oxidative Damage to Proteins and Lipids in Healthy Children, Adults, and the Elderly**," *Oxid. Med. Cell.*

Longev., vol. 2019, doi: 10.1155/2019/4393460.

[19]A. M. Uwitonze and M. S. Razzaque, "**Role of magnesium in vitamin dactivation and function**," *J. Am. Osteopath. Assoc.*, vol. 118, no. 3, pp. 181–189, 2018, doi: 10.7556/jaoa.2018.037.

[20]S. Pilz *et al.*, "**Vitamin D testing and treatment : a narrative review of current evidence**," *Endocr. Connect.*, vol. 8, pp. 27–43, 2019.

[21]M. Bilewicz-stebel, B. Bergler-czop, K. Sierant, and D. Wcisło-Dziadecka, "**Vitamin D in dermatology Witamina D w dermatologii**," *Post N Med*, vol. XXXI (1A), pp. 55–60, 2018.

[22]O. O. Oguntibeju, "**Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links**.," *Int. J. Physiol. Pathophysiol. Pharmacol.*, vol. 11, no. 3, pp. 45–63, 2019, [Online]. Available:http://www.ncbi.nlm.nih.gov/pubmed/31333808%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC6628012.

[23] G. Z. Réus, A. S. Carlessi, R. H. Silva, L. B. Ceretta, and J. Quevedo, "**Relationship of oxidative stress as a link between diabetes mellitus andmajor depressive disorder**," *Oxid. Med. Cell. Longev.*, vol. 2019, no. Mdd, 2019, doi: 10.1155/2019/8637970.

[24] M. J. Berridge, "**Vitamin D deficiency and diabetes**," *Biochem. J.*, vol. 474, no. 8, pp. 1321–1332, 2017, doi: 10.1042/BCJ20170042.

[25] M. G. A. Ansari *et al.*, "**Vitamin D supplementation is associated with increased glutathione peroxidase-1 levels in arab adults with prediabetes**," *Antioxidants*, vol. 9, no. 2, pp. 1–10, 2020, doi: 10.3390/antiox9020118.

[26]U. Gröber and M. F. Holick, "**Diabetes prevention: Vitamin D supplementation may not provide any protection if there is no evidence of deficiency!**," *Nutrients*, vol. 11, no. 11, pp. 3–7, 2019, doi: 10.3390/nu11112651.

[27] M. Saif-Elnasr, I. M. Ibrahim, and M. M. Alkady, "**Role of vitamin D on glycemic control and oxidative stress in type 2 diabetes mellitus**," *J. Res. Med. Sci.*, vol. 22, no. 1, 2017, doi: 10.4103/1735-1995.200278.

[28] M. Rahimi-Madiseh, A. Malekpour-Tehrani, M. Bahmani, and M. Rafieian-Kopaei, "**The research and development on the antioxidants in prevention of diabetic complications**," *Asian Pac. J. Trop. Med.*, vol. 9, no. 9, pp. 825–831, 2016, doi: 10.1016/j.apjtm.2016.07.001.

[29] M. G. A. Ansari *et al.*, "**Vitamin D supplementation is associated with increased glutathione peroxidase-1 levels in arab adults with prediabetes**," *Antioxidants*, vol. 9, no. 2, pp. 1–10, 2020, doi: 10.3390/antiox9020118.